A Critical Evaluation of Multiple Sequence Alignment Programs in Aligning Domains of the Bcl-2 Family

INTRODUCTION

Multiple sequence alignments are a valuable tool in the biological sciences. They can help to determine aspects of protein structure, identify important regions for protein function, and classify proteins into families. The advent of the genomic era with the complete sequencing of multiple organisms has increased the importance of correctly aligning similar proteins both within and across species. When only two proteins need to be aligned, it is possible to compare each amino acid of one sequence to that of the other and determine the best path that will maximize the alignment of the two sequences. However, the amount of computational time that is required to perform the same analysis on a larger set of sequences limits the use of this method in generating multiple sequence alignments. Thus, numerous heuristic approaches have been developed to counter this problem. These different methods may enable the programs to perform better under one set of conditions than another. Here, I assess the abilities of five multiple sequence alignment programs – ClustalW, MultAlin, T-Coffee, MAP, and ProAlign – to properly align the Bcl-2 homology domains both within a subfamily and among the subfamilies.

Overview of Multiple Sequence Alignment Programs

Many multiple sequence alignment programs have been developed based solely on one or a combination of two widely used approaches, a progressive or iterative method. In the progressive method, originally introduced by Feng and Doolittle [1], the two most similar sequences are aligned first followed by the incorporation of more divergent sequences into the

alignment. The iterative approach, on the other hand, uses a scoring function to guide the alignment such that a higher score reflects a more biologically correct alignment [2]. Often, this requires repeat iterations of the process until there is no further optimization of the score.

The programs selected for this analysis are based on variations of the progressive method. The majority of these programs first perform pairwise comparisons of the sequences in a given set to determine their relatedness. This information is used to generate a dendrogram or guide tree that reflects the degree of similarity among the sequences. The two most closely related sequences are then aligned first, and the algorithm follows the guide tree to determine the order by which additional sequences will be incorporated. MultAlin is an iterative, progressive alignment that uses the UPGMA method to generate a guide tree [3]. However, this program generates a multiple sequence alignment by first aligning within individual clusters before aligning among the clusters. Once an initial alignment is produced, the program then gives the alignment a score that is the sum of the pairwise alignment scores. It then repeats the hierarchical clustering and continues this process until there is no further change in the dendrogram that is produced. Thus, by taking into account that some subsets of the sequences may be more similar to one another than to the other sequences in the set, MultAlin is expected to work well in data sets containing different families of proteins.

ClustalW is a progressive pairwise sequence alignment that was designed to improve the sensitivity of traditional progressive alignment programs, specifically by addressing the parameter choice problem [4]. The basis for this problem is that traditional progressive alignment algorithms selected a single weight matrix and fixed gap penalties for opening and extending gaps regardless of its position within the sequence. A single weight matrix is problematic when divergent sequences are aligned because there is less sequence identity present

and more mismatches. Determining the proper weight to give to mismatches is important in determining how the sequences are to be aligned. The second issue of having fixed values for gap opening and extension is problematic because gaps do not occur randomly in proteins. Residues within a domain or secondary structure are less likely to possess gaps than linker segments between these structural elements. ClustalW improves upon both parameters by giving different weights to sequences within a set and varying the gap penalties in a position and residue specific manner. Sequences are assigned different weights based upon their evolutionary distance relationships derived from a dendrogram generated using the Neighbor Joining program. Similar sequences get down-weighted while divergent sequences are up-weighted. In addition, gap penalties are varied based upon the likelihood of a gap being present next to each of the 20 amino acids and on the presence of loops as suggested by a string of 5 or more hydrophilic residues. Gaps that occur in loop regions are penalized less than those that occur within a secondary structure. Thus, ClustalW is expected to provide enhanced sensitivity and has become a widely used program in aligning multiple sequences.

The program T-Coffee (Tree-based Consistency Objective Function for alignment Evaluation) was designed to improve upon ClustalW by addressing the problem of "greediness" that is not addressed by ClustalW [5]. The concept of "greediness" refers to the concept that mistakes made early on in an alignment can be propagated to the rest of the alignment since the two most similar sequences are aligned first, and the rest of the alignment follows this initial alignment. To generate a better alignment, T-Coffee generates both local and global pairwise alignments among all the sequences and then builds a library that incorporates both sets of alignments. The program then aligns sequences taking into account how each sequence aligns with its closest neighbor and in relation to all other sequences. By considering information from

all sequences and not just the information from the already aligned sequences, T-Coffee hopes to avoid placing too much emphasis on the alignment of the two closest related sequences and correct mistakes that may occur early on in the alignment process.

The MAP program is a global progressive alignment method that is designed to perform well with aligning sequences of various lengths [6]. Sequences that share similar regions but in which one sequence may have a region of deletion or insertion may not align well using traditional algorithms that penalize heavily for gap openings and extensions. To counter this problem, the MAP algorithm performs pairwise alignments in which terminal gaps are not penalized and large internal gaps are assigned a low penalty. These pairwise alignments are then given a score and two sequences having the highest scores are aligned first, followed by the alignment of different clusters. This method was demonstrated to perform better than the Needleman-Wunsch algorithm for sequences of different lengths and give similar results for sequences of similar lengths.

Finally, the program ProAlign is a recently developed probabilistic multiple sequence alignment strategy that uses hidden Markov models to generate a progressive alignment [7]. The program first performs pairwise alignments and clusters the sequences using the Neighbor Joining method as in ClustalW. Unlike ClustalW, ProAlign then generates a probabilistic alignment of the two sequences at each node, tracing backwards to the root node. Once the root node is reached, the program builds a multiple sequence alignment by determining the path that maximizes the probability. In addition, the program calculates a posterior probability that reflects the reliability of the aligned region. These five programs, reflecting different algorithms for performing a progressive multiple sequence alignment, will be compared in this analysis of Bcl-2 family proteins.

Bcl-2 Family Proteins

Proteins of the Bcl-2 family function as critical regulators of cell survival and cell death (reviewed in [8]). The founding member Bcl-2 was originally identified from B-cell lymphoma, and the protein was found to aid not in cell proliferation but in cell survival. Since then, additional members have been identified based on the presence of one or more of the Bcl-2 homology (BH) domains. These BH domains mediate self-interactions or associations among different family members. Bcl-2 proteins are further divided into three subfamilies based upon their function and structure (Figure 1). The first subfamily is known as the Bcl-2 subfamily and includes members that, like Bcl-2, function to promote cell survival. Members of this family include Bcl-w, Bcl-x, Mcl-1, and Bfl-1. Bcl-2, Bcl-w, and Bcl-x are closely related and share all four BH domains, while Bfl-1 and Mcl-1 are more distantly related members and share only 2 or 3 of the BH domains, respectively (Table 1). The other two subfamilies include proteins that promote apoptosis, either directly, as is the case with the Bax subfamily, or through interactions with anti-apoptotic family members, as occurs with members of the BH3 subfamily. Bax subfamily members contain BH1, BH2, and BH3 domains and include the proteins Bax, Bak, and Bok. BH3 members, as their name suggests, possess only the BH3 domain. Aside from this region, BH3 proteins share little sequence similarity. This subfamily includes the proteins Bad, Bid, Bik, Bim, and Hrk. These three subfamilies will be used to analyze the ability of the various multiple sequence alignment programs to properly align shared BH domains under three separate conditions: 1) among closely related subfamily members, 2) among more distantly related subfamily members, and 3) among members from all three subfamilies.

METHODS

Sequence and Domain Data

Members of the Bcl-2 family were identified from the Prosite database in Swiss-Prot. The entries Bcl2_Family (accession number PS50062) and BH3 (accession number PS01259) were used to obtain the accession numbers for all family members used in this analysis. The following proteins were included: Bcl-2 subfamily – Bcl-2 (P10415), Bcl-w (Q92843), Bcl-x (Q07817), Mcl-1 (Q07820), and Bfl-1 (Q16548); Bax subfamily – Bax (Q07812) and Bak (Q16611); BH3 only subfamily – Bad (Q92934), Bid (P55957), Bim (O43521), Bik (Q13323), and Hrk (O00198). Of note, the proteins Bad and Bim were noted to be false negatives in the BH3 prosite entry. All selected sequences corresponded to human proteins. The region corresponding to the BH domains within each protein were obtained from each protein entry in the Swiss-Prot database and is shown in Table 1.

Multiple Sequence Alignment Programs and Parameters

The following websites and parameters were used to perform sequence alignments with each of the different algorithms. Unless otherwise specified, the gap opening penalty was set to 10 and gap extension penalty was set at 1. MultAlin was performed at http://prodes.toulouse.inra.fr/multalin/multalin.html using a Blosum62 matrix. ClustalW and MAP were both performed at http://searchlauncher.bcm.tmc.edu/multi-align/multi-align.html. A Blosum matrix was used in both cases; however, MAP utilized a Blosum50 matrix while a particular Blosum matrix could not be specified for ClustalW. T-Coffee was performed at http://www.ch.embnet.org/software/TCoffee.html. The matrix and gap penalty parameters could not be specified for this program. The software for the ProAlign program was downloaded from

http://evol-linux1.ulb.ac.be/ueg/ProAlign/ and run using Java on a Windows personal computer. The PAM120 matrix was used for this program.

Data Presentation and Alignment Scores

Alignment outputs from each program were copied and pasted into the BoxShade 3.21 server to generate the multiple alignment views. Residues shaded red are identical in at least half of the sequences while those in blue represent similarity. To compare the alignments among different programs, a formula was developed to calculate an alignment score. First, a box was drawn around a region showing the best alignment for each domain. Within this box, each amino acid identity was assigned a value of 1, and each similarity was given a value of 0.5. To penalize for gaps and non-aligned residues, each gapped space resulted in a deduction of 0.5, and residues that should be aligned within the marked boundary but which was lied outside the boundary was scored –1. However, no deduction was assigned to terminal gaps or gaps that spanned the entire marked region. Thus, the formula for the alignment score for a given domain is as follows: [(# of identical aa) + 0.5(# of similar aa) – 0.5(gap spaces) – (# of aa outside domain boundary)]/total number of aa in boundary x 100. This scoring method provides relative numbers in which to compare the results of the multiple sequence alignments from the various programs.

RESULTS

Alignment of closely related sequences within a subfamily

A structural view of all Bcl-2 family proteins used for these analyses is shown in Figure 1, and a table listing the BH domain boundaries for each protein is displayed in Table 1. To

determine the ability of each program to align closely related sequences from the same subfamily, two different sets of analyses were performed. First, the three Bcl-2 subfamily members Bcl-2, Bcl-w, and Bcl-x were aligned (Figure 2A-E). These three proteins share all 4 BH domains and are the most closely related among the subfamily members. In this case, four out of the five programs succeeded in aligning all 4 of the BH domains within each of the proteins. T-Coffee and ProAlign gave a slightly better alignment than that of MAP and MultAlin, but in general, the four are comparable and gave comparable alignment scores (Table 2). ClustalW, however, failed to align the BH4 domain within Bcl-w (Figure 2B). The second analysis examined the alignment of the two Bax subfamily members Bax and Bak (Figure 3A-E). These two proteins share BH1, BH2, and BH3 domains. Here, all five programs were successful in aligning all three domains. However, there were a few small differences present in a couple of the alignments. MultAlin, ClustalW, and MAP aligned all three BH domains identically and provided the best possible alignments overall (Figure 3A, B, D). T-Coffee differed in its alignment of the BH3 domain in which a gap was introduced (Figure 3C). The introduction of this gap allows for the two terminal amino acids in Bax to align with two residues in Bak; however, because these two residues are not part of the BH3 domain in Bak, it is not as biologically accurate an alignment. ProAlign performed the poorest in comparison to the other programs due to its alignment of the BH1 and BH2 domains (Figure 3E). ProAlign does not have any gaps in either sequence; however, in this case, introducing a gap in one of the sequences actually provides for a better alignment of the conserved residues within each domain. Thus, while T-Coffee and ProAlign gave slightly better alignments with the Bcl-2 subfamily, the other three programs were more successful in aligning domains of the Bax subfamily. Overall,

however, all five programs were fairly successful in aligning the BH domains when a high conservation of amino acid similarity is present in the domains.

Alignment of dissimilar sequences within a subfamily

To determine how each program would handle more distantly related protein sequences, another two sets of analyses were performed. First, the BH3 only subfamily members Bad, Bid, Bim, Bik, and Hrk were aligned (Figure 4A-E). These proteins share little sequence similarity aside from the BH3 domain. In addition, the BH3 domain is the least conserved of the four BH domains. The negative alignment scores accurately reflect this poor alignment (Table 2). In this situation, none of the programs were capable of aligning the BH3 domains present in all of the sequences. The highest number of sequences that had the BH3 domain properly aligned was three, and this was achieved by the programs ProAlign and T-Coffee (Figure 4E and 4C, respectively). Both of these programs failed to align Bad and Bim. However, considering that Bad and Bim were the two proteins that are false negatives in the ProSite database for BH3 domain proteins, this is not too surprising. MultAlin, ClustalW, and MAP all gave poor alignments. MultAlin could only align two of the BH3 domains (Figure 4A) while ClustalW failed to align any (Figure 4B). MAP, on the other hand, did give a partial alignment of three of the sequences and aligned the remaining two sequences separately (Figure 4D). However, the program introduced gaps in the partially aligned BH3 domain, and a large number of gaps were present in the overall alignment. Thus, all five programs failed to align a domain that exhibits low sequence conservation.

The second means of determining how the various programs compare in aligning domains found in dissimilar proteins is by the addition of two distantly related members to the

previous alignment of the Bcl-2 subfamily. Mcl-1 and Bfl-1 are more distant members that do not possess all four BH domains. Mcl-1 contains the first three BH domains while Bfl-1 contains only BH1 and BH2. In addition, Mcl-1 has a long N-terminal insertion (Figure 1). Thus, this analysis will allow for the examination of how the absence of domains and the presence of long insertions in a protein are handled by the various multiple sequence alignment programs. In this situation, none of the programs successfully omitted sequences that lacked a particularly domain (Figure 5A-E). Ideally, Mcl-1 and Bfl-1 should have gaps present in the alignment regions of the domains that are absent. Instead, sequences from Mcl-1 or Bfl-1 were included in the alignment of the BH3 or BH4 domains with the other proteins. MultAlin was most successful overall at aligning all domains correctly in each of the proteins (Figure 5A). MAP was also highly successful, except for the presence of a domain split in the BH3 domain (Figure 5D). Both of these programs also recognized the absence of a BH4 domain in either Bfl-1 or Mcl-1 but not both. While T-Coffee did align the four domains accurately, it failed to exclude sequences from either the BH3 or BH4 alignment in proteins that did not possess them (Figure 5C). ClustalW aligned three of the four domains correctly but failed to align the BH4 domain of Bcl-w (Figure 5B). Finally, ProAlign gave a poor alignment of all of the domains, with the introduction of gaps in the middle of the BH1 and BH3 and the misalignment of sequences in BH2 and BH4 (Figure 5E). Thus, the addition of more distantly related proteins adversely affects the multiple sequence alignments for a given set of closely related proteins, and the absence of domains in a protein is not well discriminated by the programs.

Multiple sequence alignment across subfamilies

To determine whether each alignment program is capable of aligning shared domains in a large set of sequences containing proteins that share one of more domains, all Bcl-2 proteins except for Mcl-1 were aligned using each program. Mcl-1 was excluded from this analysis due to a lack of sufficient computer memory required to align this sequence using the ProAlign software. In this situation, the MAP alignment gave the highest alignment score (Table 2). The BH1, BH2, and BH4 domains are well aligned with the absence of extraneous sequences from proteins that lack these domains (Figure 6D). However, the BH3 domain is poorly aligned, with only half of the sequences containing this domain being aligned. In addition, this program produces an alignment that is filled with gaps among the sequences. However, it is of interest to note that both the Bcl-2 and Bax subfamily members are aligned closely with one another and the majority of gaps are present in the BH3 only proteins, which share little sequence similarity. T-Coffee also produced a good alignment of each of the four domains (Figure 6C). However, unlike MAP, it tries to align some sequences that do not contain a particular domain among the others that do. MultAlin aligns the BH1, BH2, and BH4 domains well but misaligns some sequences within the BH3 domain (Figure 6A). This program also includes sequences that do not possess a particular domain among those that do. This same pitfall occurs in the ClustalW alignment (Figure 6B). However, this program aligns only the BH1 and BH2 domains accurately and fails in aligning some sequences in the BH3 and BH4 domains. ProAlign was capable of aligning the BH1 and BH4 domains correctly but did not fully align the BH2 and BH3 domains (Figure 6E).

DISCUSSION

An evolutionarily correct or biologically relevant multiple sequence alignment is a very useful tool to have in molecular biology. Because of its many applications, algorithms for performing multiple sequence alignments have been developed and improved upon throughout the years. Due to a lack of sufficient computer memory and a desire for speed, heuristic approaches have largely been used in these methods. Many of these are based on a progressive pairwise alignment that aligns the two most similar sequences first followed by more distantly related sequences. In this analysis of the multiple alignment programs MultAlin, ClustalW, T-Coffee, MAP, and ProAlign, the BH domains in the Bcl-2 proteins were aligned as separate test sets. Specifically, areas of improvements in the alignment programs could be seen from this data. In general, for sequences that are closely related, such as the Bcl-2, Bcl-w, and Bcl-x, all of the programs performed well. Because these sequences shared a high degree of similarity for all shared BH domains, it provides the simplest test of aligning domains. Slight differences in the alignment of some of the BH domains could be seen, but overall, most were identical or highly similar. This was evident by the similar alignment scores calculated for the programs in aligning closely related Bcl-2 subfamily members or Bax subfamily members. Differences in the performance of the programs, however, could be found either when sequences with very low amino acid similarity were aligned or when more distantly related sequences were introduced to a set of closely related sequences. In the case of aligning the BH3 subfamily members, which is a poorly conserved domain and in which members share little sequence similarity apart from this domain, all programs performed poorly. None of the programs were capable of aligning this domain among all the members. The best alignments were produced with ProAlign and T-Coffee with the misalignment of the two proteins Bad and Bim. These two proteins possessed

less conserved BH3 domains and were noted as false positives in the ProSite database. In addition, the inclusion of more distantly related members into the Bcl-2 subfamily alignment lowered the ability of some programs to properly align all the domains. This was especially apparent with the ProAlign program, which gave a poor alignment of all four BH domains. Finally, when a large set of sequences containing members from all three subfamilies having low similarity among some of the proteins aside from a shared BH domain were aligned, most programs performed poorly. In general, similar pitfalls were present as seen previously. T-Coffee and MAP performed the best in aligning the domains. Whereas T-Coffee correctly included all proteins in each domain that possessed the domain, it also included sequences from proteins that lacked the domain. MAP succeeded in leaving out sequences that lacked a particular domain from the alignment of that domain but failed in aligning members of the BH3 subfamily with the BH3 domains within the other proteins. Thus, a few points can be noted from these different analyses. First, high amino acid conservation will provide good alignments with any of the programs. Second, the problem of aligning multiple sequences with low conservation or that are distantly related still poses a significant problem for these alignment programs. In particular, programs had difficulty in excluding sequences from a domain alignment when that domain was not present in the sequence. Thus, sequences with low amino acid similarity or which do not possess the same domains remain a problem for these various alignment programs.

Finally, some of the strengths and weaknesses of the five programs could be observed in how they handled each sequence set. ProAlign performed best with sequences that were closely related and were of similar lengths. It performed poorly when distantly related members of the Bcl-2 subfamily were introduced because these proteins possessed deletions or insertions in the sequence compared with Bcl-2. Such differences in lengths may not work well with hidden

Markov models. ClustalW performed well with highly conserved domains, such as those found in the Bcl-2 and Bax subfamilies but failed in aligning the less well conserved BH3 domain of BH3 subfamily members. T-Coffee, which was designed to improve upon ClustalW, does show an improvement. In all cases, T-Coffee generated an alignment that was as good or better than that of ClustalW. It was better at handling domains that were not well conserved as well as handling distantly related protein sequences. MultAlin and MAP, like ClustalW, performed well under conditions where the domains were well conserved but not in the case of the less conserved BH3 domain of the BH3 only subfamily. However, it was also apparent that MAP has a tendency to introduce a high number of gaps in the alignments. This can be attributed to the MAP algorithm that has a very low penalty for gaps and is designed to align conserved regions in distantly related proteins. In the analysis that included all Bcl-2 subfamilies, MAP was highly successful in excluding sequences that did not possess a particular domain from the domain alignment. This was not observed in the other programs. In conclusion, the choice of which multiple alignment program to use becomes an issue when an alignment is to be performed with sequences that have low amino acid similarity or with less conserved domains. While T-Coffee generally performed the best overall, some programs gave a slight advantage under certain conditions. It is clear that improvements on multiple sequence alignment programs are still needed to make this a better tool for molecular biology.

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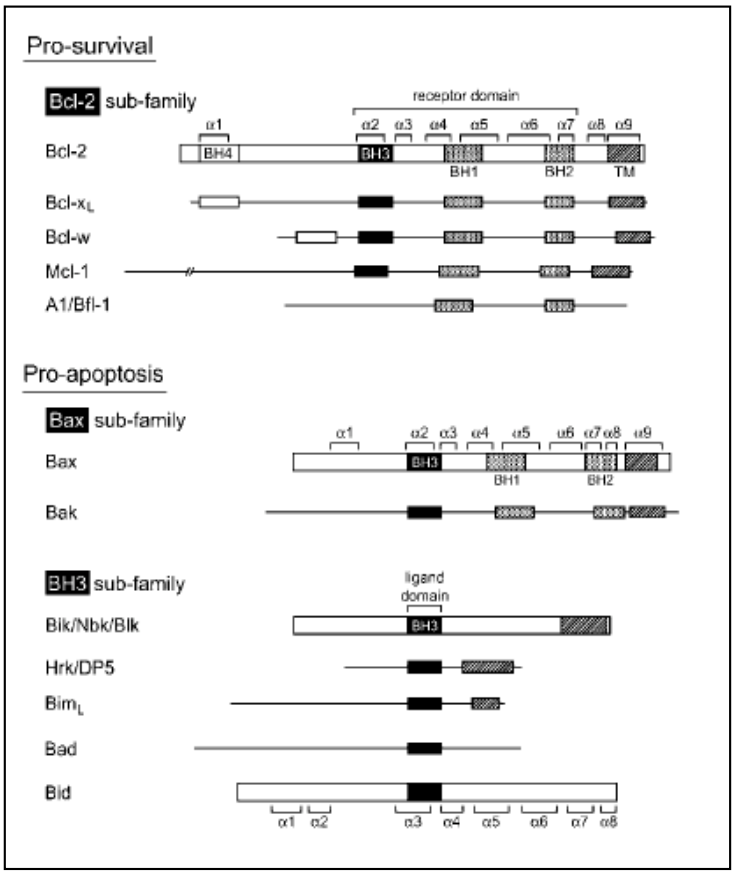


Figure 1. Structure and classification of the Bcl-2 family members selected for this analysis. (Adapted from [8])

	BH1	BH2	BH3	BH4
Bcl-2 Subfamily				
Bcl-2	136-155	187-202	93-107	10-30
Bcl-w	85-104	136-151	42-56	9-29
Bcl-x	129-148	180-195	86-100	4-24
Mcl-1	252-272	304-319	209-223	
Bfl-1	77-97	132-147		
Bax Subfamily				
Bax	98-118	150-165	59-73	
Bak	117-136	169-184	74-88	
BH3 Subfamily				
Bik			57-71	
Bid			86-100	
Hrk			33-47	
Bad			110-124	
Bim			148-162	

Table 1. BH domains in Bcl-2 family proteins. The locations of all known BH domains for each Bcl-2 family member are shown (from Swiss-Prot).

	MultAlin	ClustalW	T-Coffee	MAP	ProAlign
Bcl-2 (3)					
BH1	88.33	88.33	88.33	88.33	88.33
BH2	72.92	72.92	72.92	72.92	72.92
BH3	75.00	75.55	75.55	75.55	75.55
BH4	70.49	19.05	72.95	70.49	72.95
Total	306.74	255.85	309.75	307.29	309.75
Bcl-2 All					
BH1	73.04	73.04	73.04	73.04	28.51
BH2	62.35	62.35	62.35	62.35	6.58
BH3	36.49	37.33	37.33	27.97	13.33
BH4	46.25	20.49	36.08	53.79	10.48
Total	218.13	193.21	208.80	217.15	58.90
Bax					
BH1	56.98	56.98	56.98	56.98	47.62
BH2	42.42	42.42	42.42	42.42	40.63
BH3	39.66	39.66	36.21	39.66	39.66
Total	139.06	139.06	135.61	139.06	127.91
BH3					
BH3	-41.10	-59.70	-18.46	-51.32	-8.67
All					
BH1	26.03	24.18	35.15	61.89	27.72
BH2	20.22	17.37	34.33	50.00	10.97
BH3	-16.26	-13.11	25.00	-38.89	-2.78
BH4	29.39	15.09	20.70	48.00	-9.50
Total	59.38	43.53	115.18	121.00	26.41

Table 2. Alignment scores for the various algorithms in each set of analyses. See methods for how these values were calculated. The total score is calculated by adding the individual scores of each domain for a given algorithm. "Bcl-2 (3)" refers to the alignment of Bcl-2, Bcl-w, and Bcl-x whereas "Bcl-2 All" includes Mcl-1 and Bfl-1. "All" refers to the alignment of all selected Bcl-2 family proteins with the exception of Mcl-1.

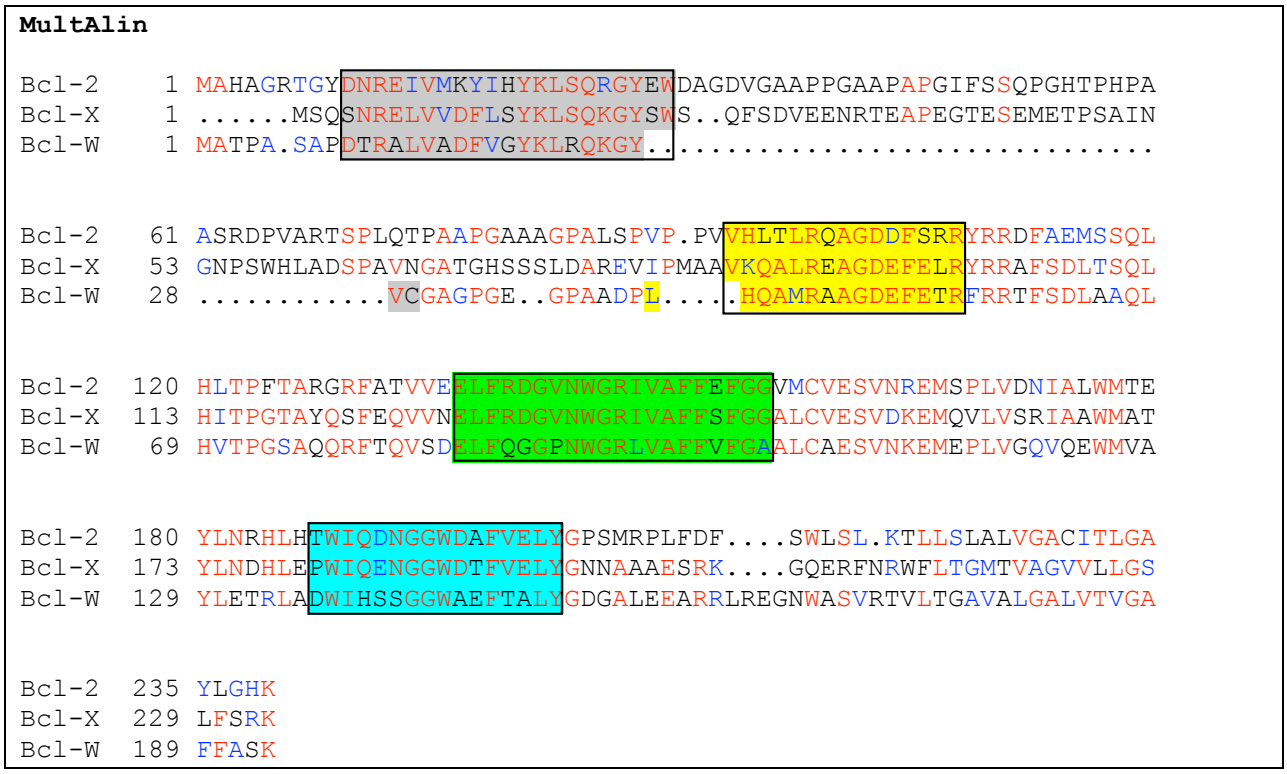
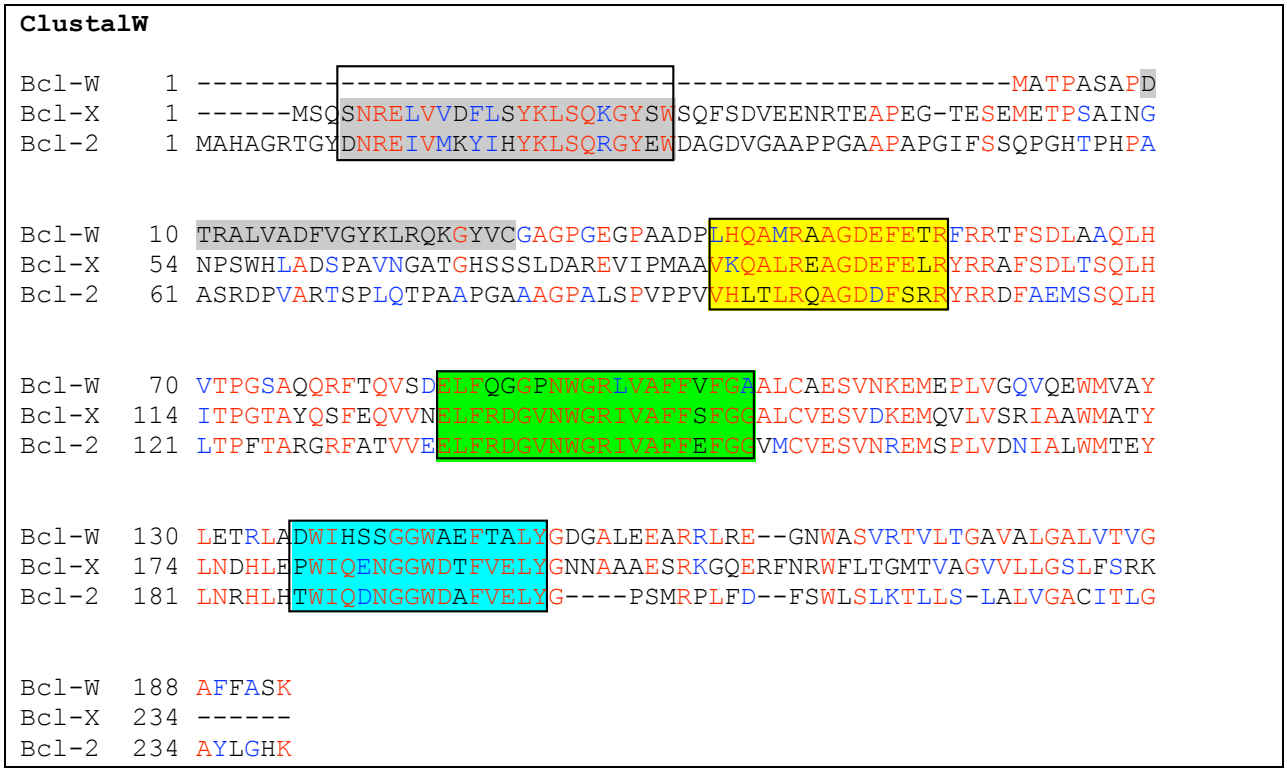


Figure 2A.



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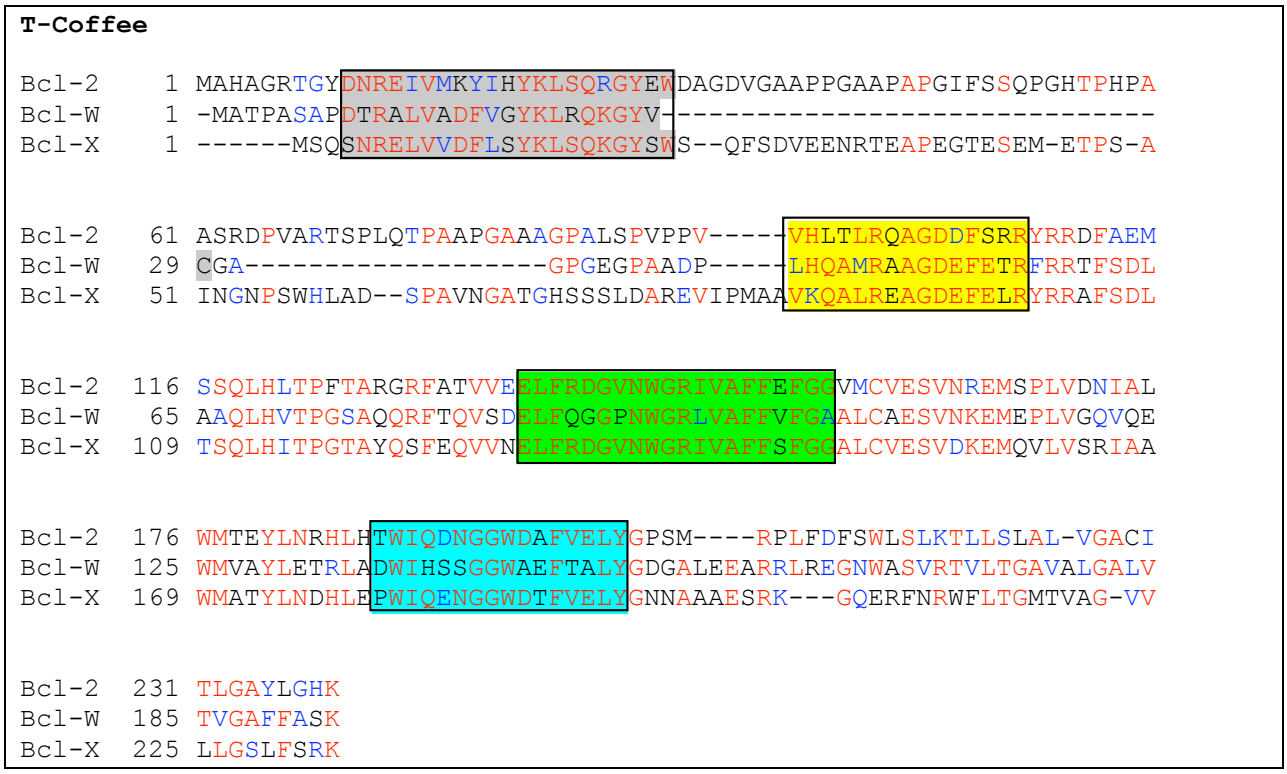


Figure 2C.

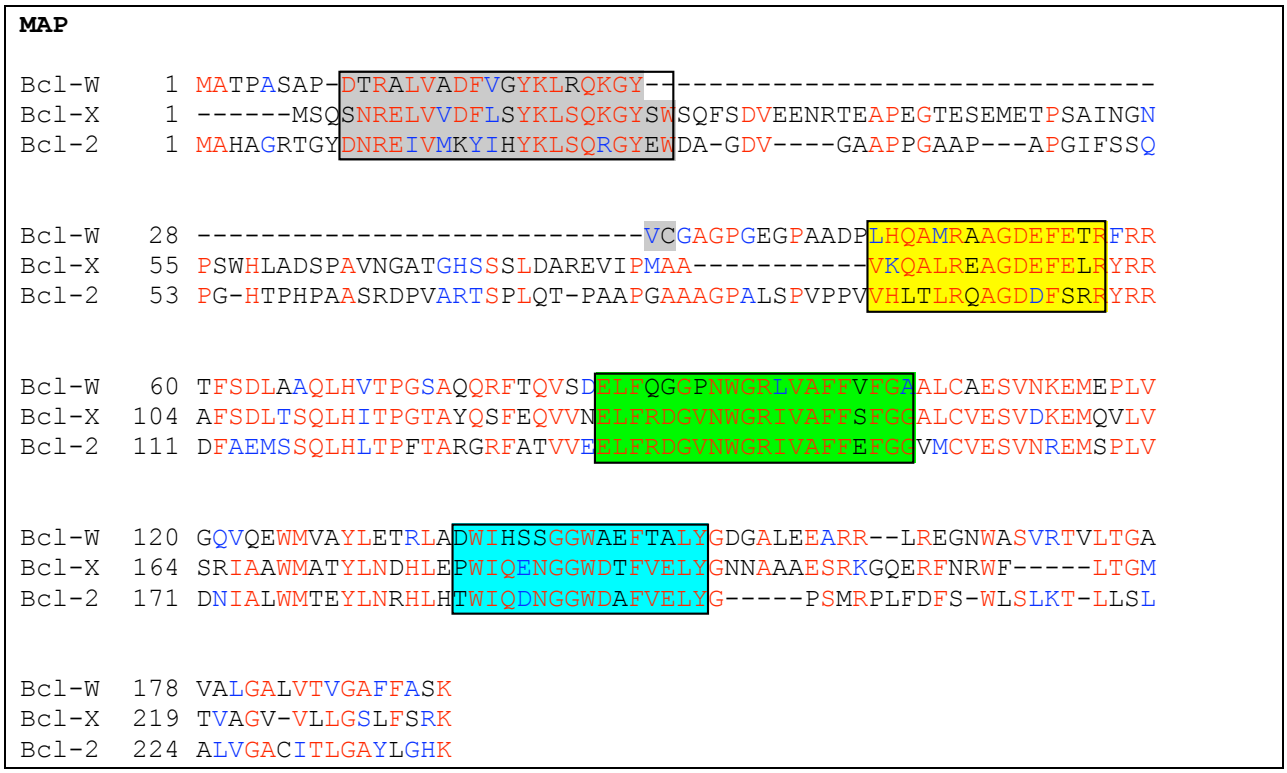


Figure 2D.

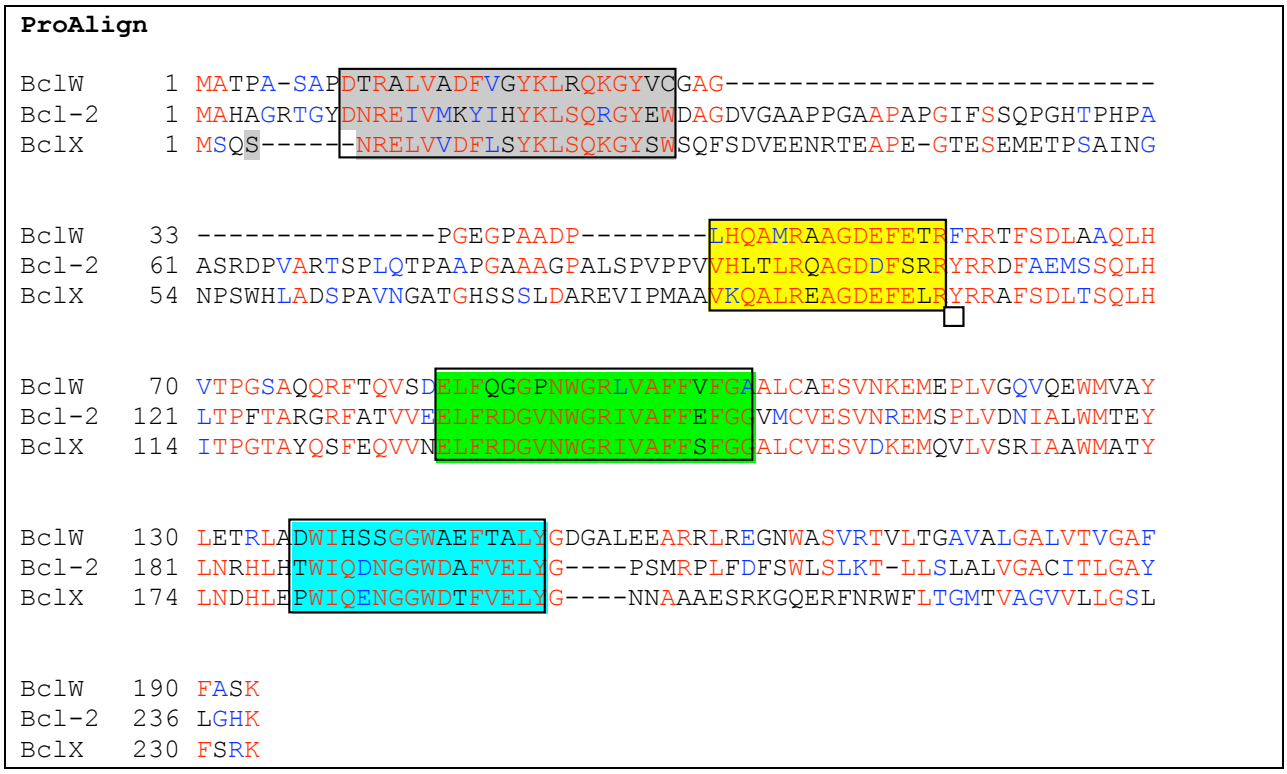

ProAlign
BclW 1 MATPA-SAPDTRALVADFVGYKLRQKGYVCGAG----------
Bcl-2 1 MAHAGRTGYDNREIVMKYIHYKLSQRGYEWDAGDVGAAPPGAAPAPGIFSSQPGHTPHPA
BclX 1 MSQS------NRELVVDFLSYKLSQKGYSWSQFSDVEENRTEAPE-GTESEMETPSAING
BclW 33 ----------PGEGPAADP--------LHQAMRAAGDEFETRFRRTFSDLAAQLH
Bcl-2 61 ASRDPVARTSPLQTPAAPGAAAGPALSPVPPVVHLTLRQAGDDFSRRYRRDFAEMSSQLH
BclX 54 NPSWHLADSPAVNGATGHSSSLDAREVIPMAAVKQALREAGDEFELRYRRAFSDLTSQLH
BclW 70 VTPGSAQQRFTQVSDELFQGGPNWGRLVAFFVFGAALCAESVNKEMEPLVGQVQEWMVAY
Bcl-2 121 LTPFTARGRFATVVEELFRDGVNWGRIVAFFEFGGVMCVESVNREMSPLVDNIALWMTEY
BclX 114 ITPGTAYQSFEQVVNELFRDGVNWGRIVAFFSFGGALCVESVDKEMQVLVSRIAAWMATY
BclW 130 LETRLADWIHSSGGWAEFTALYGDGALEEARRLREGNWASVRTVLTGAVALGALVTVGAF
Bcl-2 181 LNRHLHTWIQDNGGWDAFVELYG----PSMRPLFDFSWLSLKT-LLSLALVGACITLGAY
BclX 174 LNDHLEPWIQENGGWDTFVELYG----NNAAAESRKGQERFNRWFLTGMTVAGVVLLGSL
BclW 190 FASK
Bcl-2 236 LGHK
BclX 230 FSRK

Figure 2E.

MultAlin

```
BAK     1 MASGQGPGPPRQECGEPALPSASEEQVAQDTEEVFRSYVFYRHQQEQEAEGVAAPADPEM
BAX     1 .MDGSGEQP...RGGGPT....SSEQIMKTGALLLQGFI.......QDRAGRMGGEAPEL

BAK    61 VTLPLQPSSTMGQVGRQLAIIGDDINRRYDSEFQTMLQHLQPTAENAYEYFTKIATSLFE
BAX    46 ALDPVPQDASTKKLSECLKRIGDELDS..NMELQRMIAAVD..TDSPREVFFRVAADMFS

BAK   121 SG..INWGRVVALLGFGYRLALHVYQHGLTGFLGQVTRFVVDFMLHHCIARWIAQRGGWVA
BAX   102 DGNFNWGRVVALFYFASKLVLKALCTKVPELIRTIMGWTLDFLRERLLG..WIQDQGGWDG

BAK   180 ALNLGNGPILN.VLVVLGVVLLGQFVVRRFFKS
BAX   161 LLSYFGTPTWQTVTIFVAGVLTASLTIWKKMG.
```

Figure 3A.

ClustalW

```
BAK     1 MASGQGPGPPRQECGEPALPSASEEQVAQDTEEVFRSYVFYRHQQEQEAEGVAAPADPEM
BAX     1 -MDGSGEQPRG---GGPTS-----------SEQIMKTGALLLQGFIQDRAGRMGGEAPEL

BAK    61 VTLPLQPSSTMGQVGRQLAIIGDDINRRYDSEFQTMLQHLQPTAENAYEYFTKIATSLFE
BAX    46 ALDPVPQDASTKKLSECLKRIGDELDS--NMELQRMIAAVDTDSP--REVFFRVAADMFS

BAK   121 SG-INWGRVVALLGFGYRLALHVYQHGLTGFLGQVTRFVVDFMLHHCIARWIAQRGGWVA
BAX   102 DGNFNWGRVVALFYFASKLVLKALCTKVPELIRTIMGWTLDFLRERLLG-WIQDQGGWDG

BAK   180 ALNLGNGPILNVLVVLGVVLLGQFVVRRFFKS
BAX   161 LLSYFGTPTWQTVTIFVAGVLTASLTIWKKMG
```

Figure 3B.

T-Coffee

```
BAK     1 MASGQGPGPPRQECGEPALPSASEEQVAQDTEEVFRSYVFYRHQQEQEAEGVAAPADPEM
BAX     1 -MDGSGEQP---RGGG---PTSSEQ-IMKTGALLLQGFI-------QDRAGRMGGEAPEL

BAK    61 VTLPLQPSSTMGQVGRQLAIIGDDINRRYDSEFQTMLQHLQPTAENAYEYFTKIATSLFE
BAX    46 ALDPVPQDASTKKLSECLKRIGDEL----DSNMELQRMIAAVDTDSPREVFFRVAADMFS

BAK   121 SG-INWGRVVALLGFGYRLALHVYQHGLTGFLGQVTRFVVDFMLHHCIARWIAQRGGWVA
BAX   102 DGNFNWGRVVALFYFASKLVLKALCTKVPELIRTIMGWTLDFLRERLLG-WIQDQGGWDG

BAK   180 ALNLGNGPILNVLVVL--GVVLLGQFVVRRFFKS
BAX   161 LLSYFGTPTWQTVTIFVAGVLTASLTIWKKMG--
```

Figure 3C.

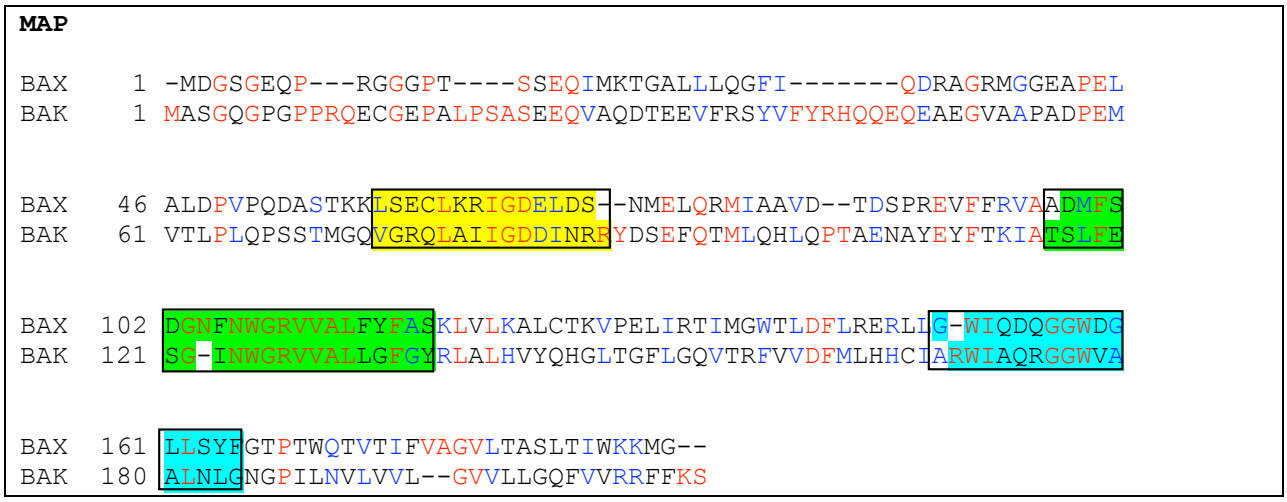


Figure 3D.

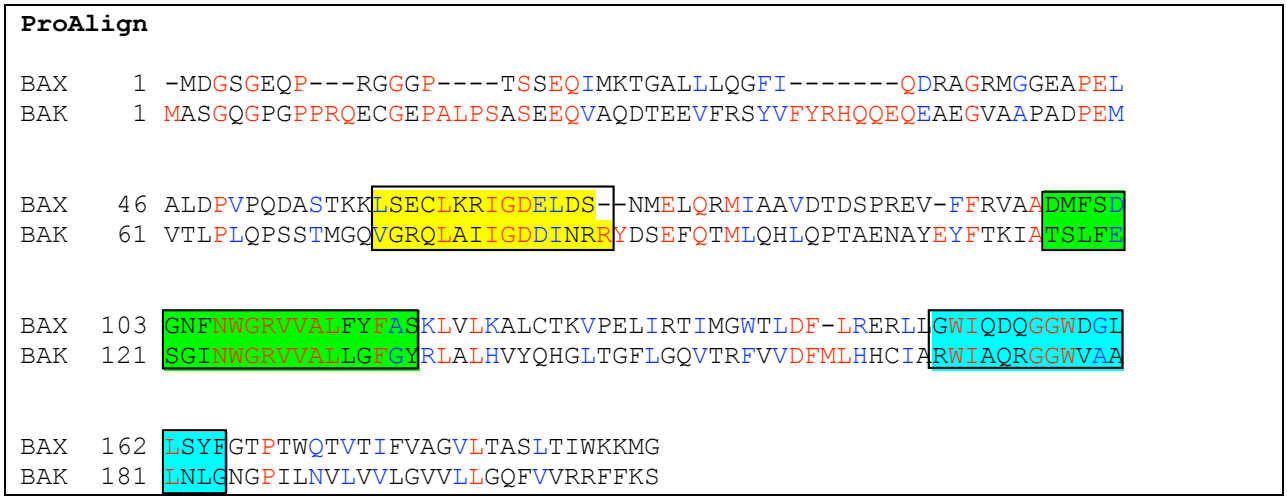


Figure 3E.

MultAlin

```
BAD     1 ..................MFQIPEFEPSEQEDS..SSAERGLGPSPAGDGPSGSGKHHRQA
BID     1 MDCEVNNGSSLRDECITNLLVFGFLQSCSDNS.FRRELDALGHELPVLAPQWEGYDELQT
BIM     1 MAKQPSDVSSECDREGRQLQPAERPPQLRPGAPTSLQTEPQGNPEGNHGGEGDSCPHGSP
BIK     1 ..................................MSEVRPLSRDILMETLLYEQLLEPPT
HRK     1 ........................................MCPCPLHRGRGPPAVCACS

BAD    42 PGLLWDA.SHQQEQPTSSSHHGGAGAVEIRSRHSSYPAGTEDDEGMGEEPSPFRGRSRSA
BID    60 DGNR..S.SHSRLGRIEADSESQEDIIRNIARHLAQVGD.SMDRSI..PPGLVNGLALQL
BIM    61 QGPL..A.PPASPGPFATRSPLFIFMRRSSLLSRSSSGYFSFDTDR..SPAPMS.CDKST
BIK    27 MEVLGMTDSEEDLDPMEDFDSLECMEGSDALALRLACIGDEMDVSLRAPRLAQLSEVAMH
HRK    20 AGRLGLRSSAAQLTAARLKALGDELHQRTMWRRRARSRRAPAPGALPTYWPWLCAAAQVA

BAD   101 PPNLWAAQRYGRELRRMSDEFVDSFKKGLPRPKS.AGTATQMRQ..SSSWTRVFQSWWDR
BID   114 RNTSRSEEDRNRDLATALEQLLQAYPRDMEKEKTMLVLALLLAKKVASHTPSLLRDVFHT
BIM   115 QTPSPPCQAFNHYLS.AMASMRQAEPADMRPE...IWIAQELRRIGDEFNAYYARRVFLN
BIK    87 SLGLAFIYDQTEDIRDVLRSFMDGFTTLKEN......IMRFWRSPNPGSWVSCEQVLLAL
HRK    80 ALAAWLLGRRNL.............................................

BAD   158 NLGRGSSAPSQ.................
BID   174 TVNFINQNLRTYVRSLARNGMD......
BIM   171 NYQAAEDHPRMVILRLLRYIVRLVWRMH
BIK   141 LLLLA......LLLPLLSGGLHLLLK..
HRK       ..........................
```

Figure 4A.

ClustalW

```
BIK     1 -----------MSEVRPLSRDILMETLLYEQLLEPPTMEVLGMTDSEEDLDPMEDFDSLE
HRK     1 ------------------------------------------------------------MC
BID     1 ---MDCEVNNGSSLRDECITNLLVFGFLQSCSDNSFRRELDALGHELPVLAPQWEGYDEL
BAD     1 ------MFQIPEFEPSEQEDSSSAERGLGPSPAGDGPSGSGKHHRQAPGLLWDASHQQEQ
BIM     1 MAKQPSDVSSECDREGRQLQPAERPPQLRPGAPTSLQTEPQGNPEGNHGGEGDSCPHGSP

BIK    50 CMEGSDALALRLACIGDEMDVSLRAPR-----LAQLSEVAMHSLGLAFIYDQTEDIRDVL
HRK     3 PCPLHRGRGPPAVCACSAGRLGLRSS-------AAQLTAARLKALGDELHQRTMWRR----
BID    58 QTDGNRSSHSRLGRIEADSESQEDIIRNIARHLAQVGDSMDRSIPPGLVNGLALQLRNTS
BAD    55 PTSSSHHGGAGAVEIRSRHSSYPAGTEDDEGMGEEPSPFRGRSRSAPPNLWAAQRYGREL
BIM    61 QGPLAPPASPGPFATRSPLFIFMRRSSLLSRSSSGYFSFDTDRSPAPMSCDKSTQTPSPP

BIK   105 RSFMD---GFTTLKENIMRFWRSPNPGSWVSCEQVLLALLLLALLLPLLSGGLHLLLK-
HRK    53 R-----------------ARSRRAPAPGALPTYWPWLCAAAQVAALAAWLLGRRNL-----
BID   118 RSEEDRNRDLATALEQLLQAYPRDMEKEKTMLVLALLLAKKVASHTPSLLRDVFHTTVNF
BAD   115 RRMSD-----EFVDSFKKGLPRPKSAGTATQMRQSSSWTRVFQSWWDRNLGRGSSAPSQ-
BIM   121 CQAFN------HYLSAMASMRQAEPADMRPEIWIAQELRRIGDEFNAYYARRVFLNNYQA
```

```
BIK       ------------------------
HRK       ------------------------
BID   178 INQNLRTYVRSLARNGMD------
BAD       ------------------------
BIM   175 AEDHPRMVILRLLRYIVRLVWRMH
```

Figure 4B.

T-Coffee

```
BAD     1 MFQIPEFEPSEQEDSS-------------------------------------------S
BID     1 -------MDCEVNNGSSLRDECITNL--------------------------------L-
BIK     1 -----------MSEVRPLSRDILMET--------------------------------LL
BIM     1 MAKQP----SDVSSECDREGRQLQPAERPPQLRPGAPTSLQTEPQGNPEGNHGGEGDSCP
HRK     1 ------------------------MCP-------------------------------CP

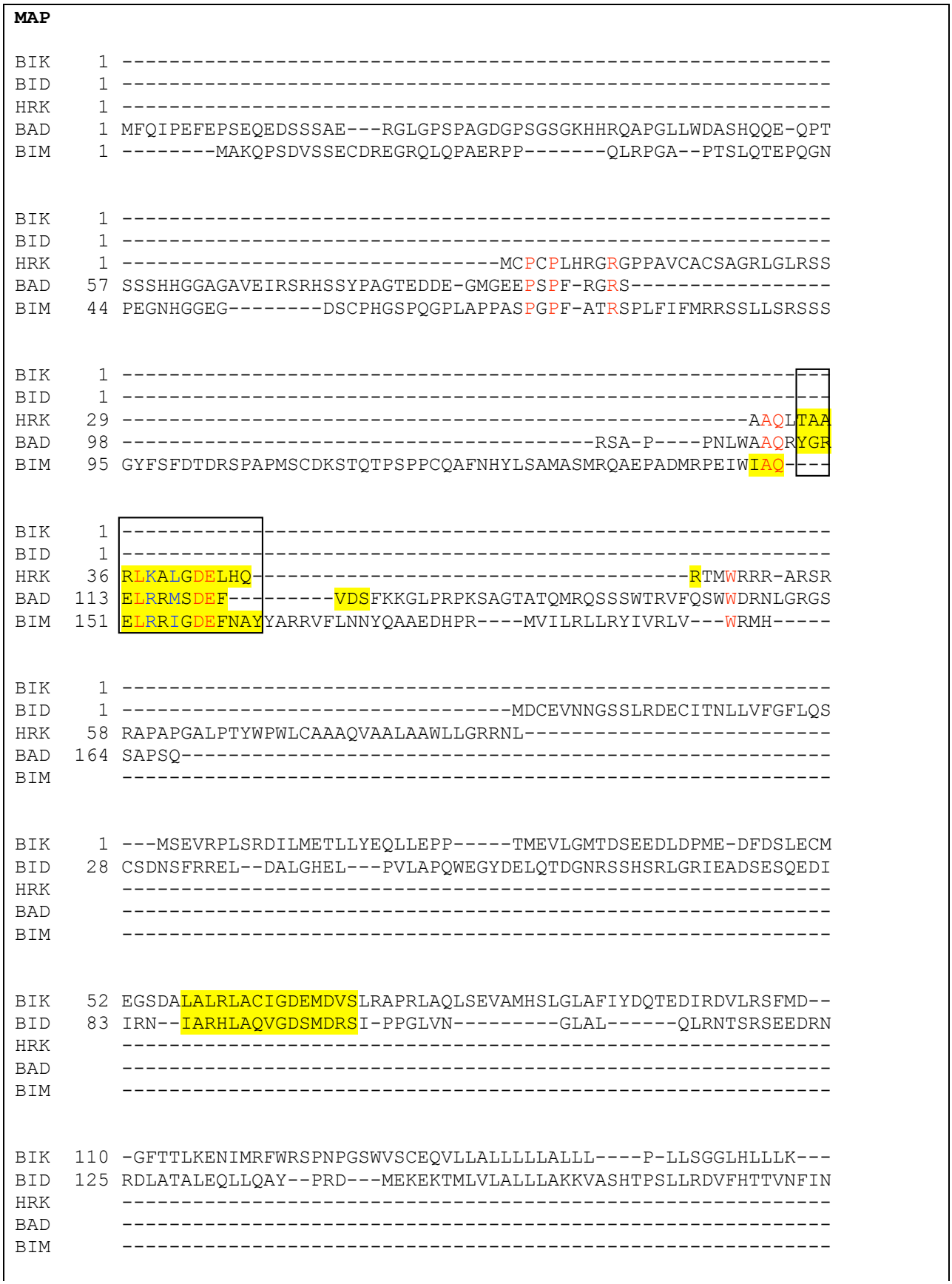

BAD    18 AERGLGP--SPAGDG------PSGSGKHHRQAPGL---------LWDASHQQEQPTSSSH
BID    21 --------------V-FGFLQSCSDNSFRRELDALGHELPVLAPQWEGYDELQTDGNRSS
BIK    18 YEQLLEP---PTMEV-LGMTDSEEDLDPMEDFDSL------ECME--------------G
BIM    57 HGSPQGPLAPPASPGPFATRS-----------------------------------PL
HRK     6 LHRGRGP---PAV-----------------------------CACSAG-----RLGLRSS


BAD    61 HGGAGAVEIRSRHSS-----------YPAGTEDDEGMGEEPSPFRGRS----RSAPPNLWAAQ
BID    66 HSRLGRIEADSESQEDIIRNIARHLAQVGDSMDRSI----------------PPGL----
BIK    54 SDA----------------LALRLACIGDEMDVSLRAPRLAQLSEVAMHSLGLAF----
BIM    80 FIFMRRSSLLSRS-------SSGYFSFDT-----------------DRSPAPMS----
HRK    29 AAQL---------------TAARLKALGDELHQ-------------------------


BAD   109 RYGR--ELRRMSDEFVDSF-------KK------GLPRPKSAGTATQMRQSSSWTRVFQS
BID   106 VNGLALQLRNTSRSEEDRNRDLATALEQLLQAYPRD------MEKEKT----MLVLALLL
BIK    93 IYDQTEDIRDVLRSFMDGF----TTLKENIMRFWRSPNPGSWVSCEQVLLALLLLLALLL
BIM   110 CDKSTQTPSPPCQAFNHY----LSAMASMRQAEPADMRPEIWIAQE-------------L
HRK    47 ----RTMWRRRARS-------------------RRAPAPGALPTYWPWLCAAAQVAALAA


BAD   154 -WWD-----RNLGRGSSAPSQ------------------------
BID   156 AKKVASHTPSLLRDVFHTTVNFINQNLRTYVRSLARNGMD------
BIK   149 ---------PLLSGGLHLLLK------------------------
BIM   153 RRIGDEFNAYYARRVFLNNYQAAEDHPRMVILRLLRYIVRLVWRMH
HRK    84 ---------WLLGRRNL----------------------------
```

Figure 4C.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BID 1 ----------
HRK 1 ----------
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BIM 1 --------MAKQPSDVSSECDREGRQLQPAERPP-------QLRPGA--PTSLQTEPQGN
BIK 1 ----------
BID 1 ----------
HRK 1 ----------MCPCPLHRGRGPPAVCACSAGRLGLRSS
BAD 57 SSSHHGGAGAVEIRSRHSSYPAGTEDDE-GMGEEPSPF-RGRS----------
BIM 44 PEGNHGGEG--------DSCPHGSPQGPLAPPASPGPF-ATRSPLFIFMRRSSLLSRSSS
BIK 1 ----------
BID 1 ----------
HRK 29 ----------AAQLTAA
BAD 98 ----------RSA-P----PNLWAAQRYGR
BIM 95 GYFSFDTDRSPAPMSCDKSTQTPSPPCQAFNHYLSAMASMRQAEPADMRPEIWIAQ----
BIK 1 ----------
BID 1 ----------
HRK 36 RLKALGDELHQ----------RTMWRRR-ARSR
BAD 113 ELRRMSDEF---------VDSFKKGLPRPKSAGTATQMRQSSSWTRVFQSWWDRNLGRGS
BIM 151 ELRRIGDEFNAYYARRVFLNNYQAAEDHPR----MVILRLLRYIVRLV---WRMH-----
BIK 1 ----------
BID 1 ----------MDCEVNNGSSLRDECITNLLVFGFLQS
HRK 58 RAPAPGALPTYWPWLCAAAQVAALAAWLLGRRNL----------
BAD 164 SAPSQ----------
BIM ----------
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BID 28 CSDNSFRREL--DALGHEL---PVLAPQWEGYDELQTDGNRSSHSRLGRIEADSESQEDI
HRK ----------
BAD ----------
BIM ----------
BIK 52 EGSDALALRLACIGDEMDVSLRAPRLAQLSEVAMHSLGLAFIYDQTEDIRDVLRSFMD--
BID 83 IRN--IARHLAQVGDSMDRSI-PPGLVN---------GLAL------QLRNTSRSEEDRN
HRK ----------
BAD ----------
BIM ----------
BIK 110 -GFTTLKENIMRFWRSPNPGSWVSCEQVLLALLLLLALLL----P-LLSGGLHLLLK---
BID 125 RDLATALEQLLQAY--PRD---MEKEKTMLVLALLLAKKVASHTPSLLRDVFHTTVNFIN
HRK ----------
BAD ----------
BIM ----------

```
BIK       ----------------
BID   180 QNLRTYVRSLARNGMD
HRK       ----------------
BAD       ----------------
BIM       ----------------
```

Figure 4D.

ProAlign

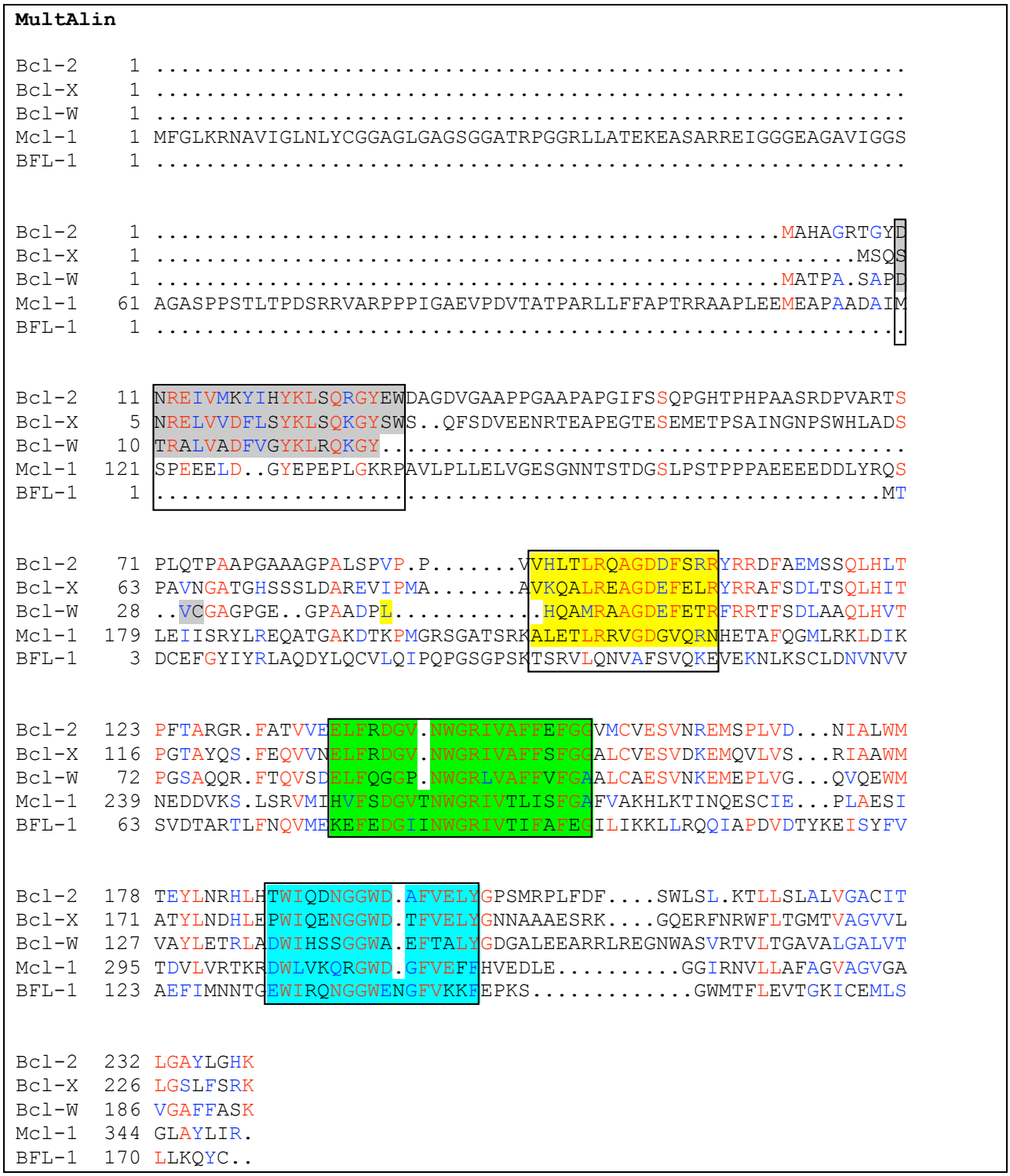
```
HRK     1 -----MCPCPLHRGRGPPAVC------ACSAGRLGLR---------------SSAAQLT-
BIK     1 ----MSEVRPLSRDILMETLLYEQLLEPPTMEVLGMT--------------DSEEDLDP
BIM     1 MAKQPSDVSSECDREGRQLQPAERPPQLRPGAPTSLQTEPQGNPEGNHGGEGDSCPHGSP
BID     1 MDCEVNNGSSLRDECITNLL-VFGFLQSCSDNSFRRELDALGHELPVLAPQWEGYDELQT
BAD     1 MFQIPEFEPSEQEDS--------------SSAERGLGPSPAGDGPSGSGKHHRQAPGLLW


HRK    34 ---------------------------AARLKALGDELHQR------------------
BIK    42 MEDFDSLECMEGSDA-----------LALRLACIGDEMDVSLRAPRLAQLS------EVA
BIM    61 QGPLAPPASPGPFATRSPLFIFMRRSSLLSRSSSGYFSFDTDRSPAPMSCDKSTQTPSPP
BID    60 DGNRSSHSRLGRIEADSESQEDIIRNIARHLAQVGDSMDRSIPPGLVNGLALQLRNTSRS
BAD    47 DASHQQEQPTSSSHHGGAGAVEIRSRHSSYPAGTEDDEGMGEEPSPFRGRSRSAPPNLWA


HRK    48 ----------------------------TMWRRRARSRRAPAPGALPTYWPWLCAAAQVA
BIK    85 MHSLGLAFIYDQTEDIRDVLRSFMDGFTTLKENIMRFWRSPNPGSWVSCEQVLLALLLLL
BIM   121 CQAFNHYLSAMASMRQAEPADMRPEIWIAQELRRIGDEFNAYYARRVFLNNYQAAEDHPR
BID   120 EEDRNRDLATALEQLLQAYPRDMEKEKTMLVLALLLAKKVASHTPSLLRDVFHTTVNFIN
BAD   107 AQRYGRELRRMSDEFVDSFKKGLPRPKSAGTATQMRQSSSWTRVFQSWWDRNLGRGSSAP


HRK    80 ALAAWLL-GRRNL-----
BIK   145 ALLLPLLSGGLHLL--LK
BIM   181 MVILRLLRYIVRLVWRMH
BID   180 QNLRTYVRSLAR--NGMD
BAD   167 SQ----------------
```

Figure 4E.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BFL-1 1 .....MT 7
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Bcl-X 5 NRELVVDFLSYKLSQKGYSWS..QFSDVEENRTEAPEGTESEMETPSAINGNPSWHLADS 63
Bcl-W 10 TRALVADFVGYKLRQKGY..... 27
Mcl-1 121 SPEEELD..GYEPEPLGKRPAVLPLLELVGESGNNTSTDGSLPSTPPPAEEEEDDLYRQS 179
BFL-1 1 20
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Bcl-X 63 PAVNGATGHSSSLDAREVIPMA.....AVKQALREAGDEFELRYRRAFSDLTSQLHIT 115
Bcl-W 28 ..VCGAGPGE..GPAADPL.....HQAMRAAGDEFETRFRRTFSDLAAQLHVT 77
Mcl-1 179 LEIISRYLREQATGAKDTKPMGRSGATSRKALETLRRVGDGVQRNHETAFQGMLRKLDIK 238
BFL-1 3 DCEFGYIYRLAQDYLQCVLQIPQPGSGPSKTSRVLQNVAFSVQKEVEKNLKSCLDNVNVV 62
Bcl-2 123 PFTARGR.FATVVEELFRDGV.NWGRIVAFFEFGGVMCVESVNREMSPLVD...NIALWM 180
Bcl-X 116 PGTAYQS.FEQVVNELFRDGV.NWGRIVAFFSFGGALCVESVDKEMQVLVS...RIAAWM 172
Bcl-W 72 PGSAQQR.FTQVSDELFQGGP.NWGRLVAFFVFGAALCAESVNKEMEPLVG...QVQEWM 129
Mcl-1 239 NEDDVKS.LSRVMIHVFSDGVTNWGRIVTLISFGAFVAKHLKTINQESCIE...PLAESI 297
BFL-1 63 SVDTARTLFNQVMEKEFEDGIINWGRIVTIFAFEGILIKKLLRQQIAPDVDTYKEISYFV 122
Bcl-2 178 TEYLNRHLHTWIQDNGGWD.AFVELYGPSMRPLFDF....SWLSL.KTLLSLALVGACIT 232
Bcl-X 171 ATYLNDHLEPWIQENGGWD.TFVELYGNNAAAESRK....GQERFNRWFLTGMTVAGVVL 225
Bcl-W 127 VAYLETRLADWIHSSGGWA.EFTALYGDGALEEARRLREGNWASVRTVLTGAVALGALVT 186
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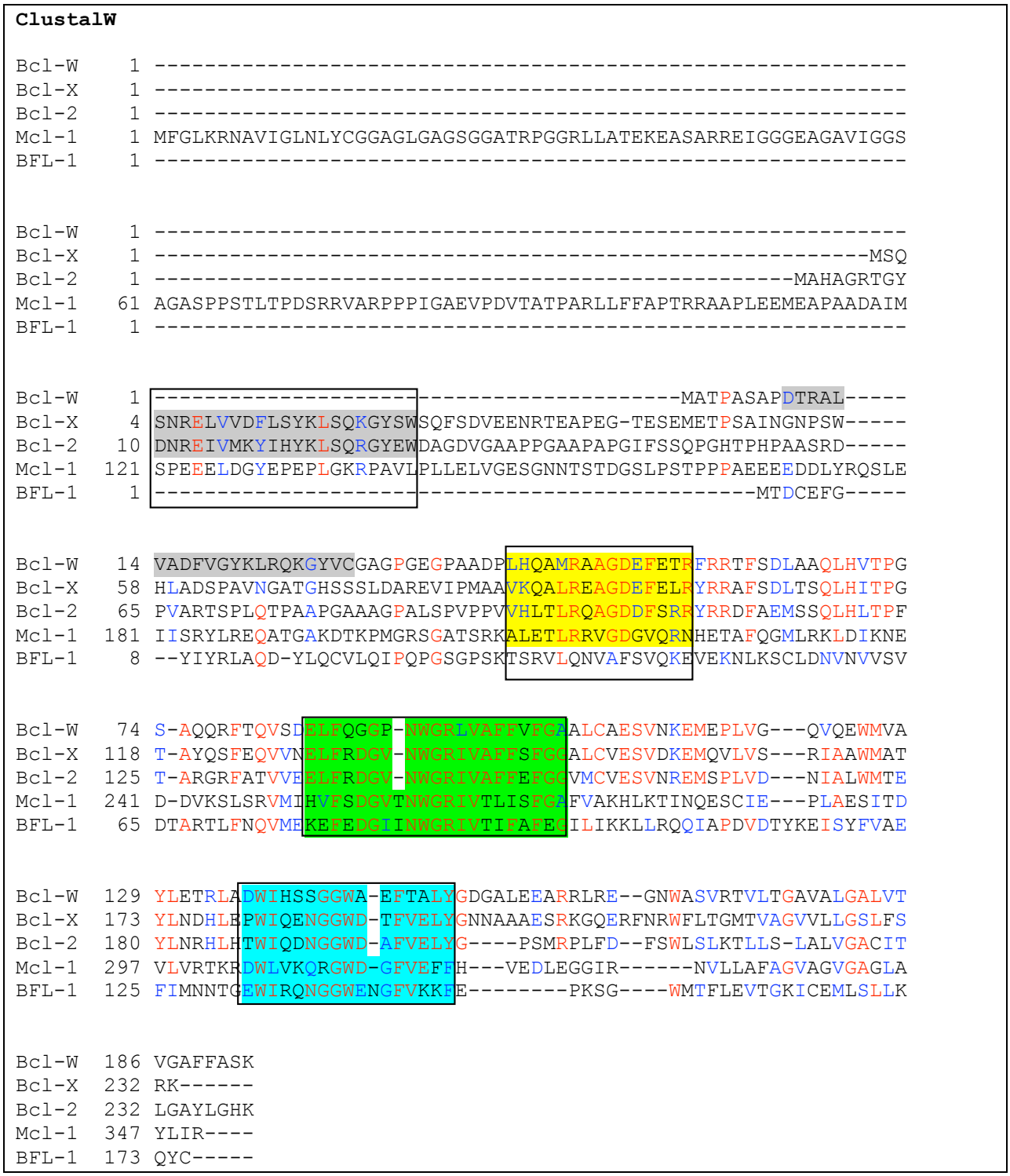
Figure 5A.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BFL-1 8 --YIYRLAQD-YLQCVLQIPQPGSGPSKTSRVLQNVAFSVQKEVEKNLKSCLDNVNVVSV
Bcl-W 74 S-AQQRFTQVSDELFQGGP-NWGRLVAFFVFGAALCAESVNKEMEPLVG---QVQEWMVA
Bcl-X 118 T-AYQSFEQVVNELFRDGV-NWGRIVAFFSFGGALCVESVDKEMQVLVS---RIAAWMAT
Bcl-2 125 T-ARGRFATVVEELFRDGV-NWGRIVAFFEFGGVMCVESVNREMSPLVD---NIALWMTE
Mcl-1 241 D-DVKSLSRVMIHVFSDGVTNWGRIVTLISFGAFVAKHLKTINQESCIE---PLAESITD
BFL-1 65 DTARTLFNQVMEKEFEDGIINWGRIVTIFAFGGILIKKLLRQQIAPDVDTYKEISYFVAE
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Bcl-X 173 YLNDHLEPWIQENGGWD-TFVELYGNNAAAESRKGQERFNRWFLTGMTVAGVVLLGSLFS
Bcl-2 180 YLNRHLHTWIQDNGGWD-AFVELYG----PSMRPLFD--FSWLSLKTLLS-LALVGACIT
Mcl-1 297 VLVRTKRDWLVKQRGWD-GFVEFFH---VEDLEGGIR------NVLLAFAGVAGVGAGLA
BFL-1 125 FIMNNTGEWIRQNGGWENGFVKKFE--------PKSG----WMTFLEVTGKICEMLSLLK
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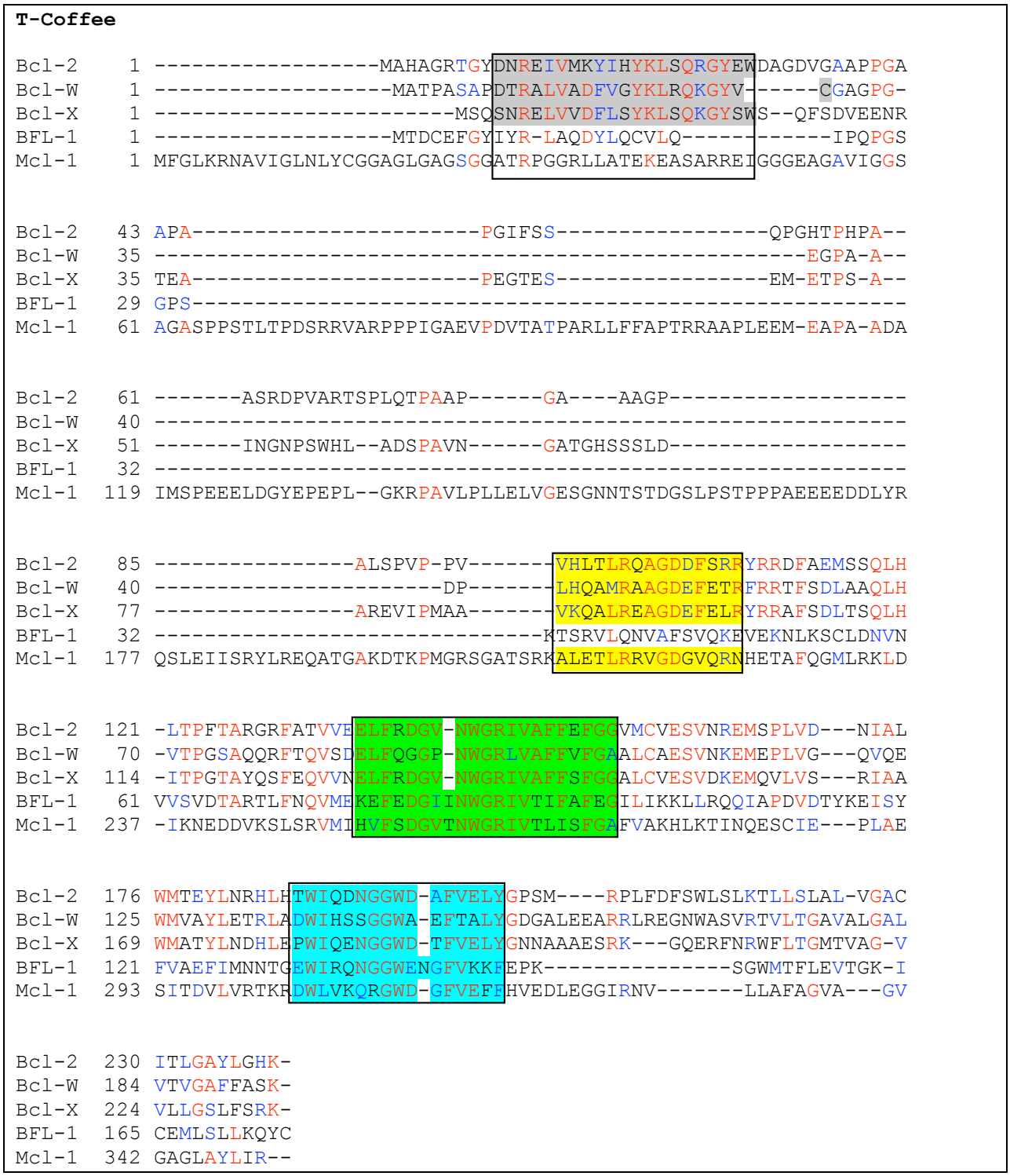
Figure 5B.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Bcl-X 1 ----------MSQSNRELVVDFLSYKLSQKGYSWS--QFSDVEENR
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Bcl-W 35 ----------EGPA-A--
Bcl-X 35 TEA----------PEGTES----------EM-ETPS-A--
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Bcl-2 85 ----------ALSPVP-PV-------VHLTLRQAGDDFSRRYRRDFAEMSSQLH
Bcl-W 40 ----------DP-------LHQAMRAAGDEFETRFRRTFSDLAAQLH
Bcl-X 77 ----------AREVIPMAA-------VKQALREAGDEFELRYRRAFSDLTSQLH
BFL-1 32 ----------KTSRVLQNVAFSVQKEVEKNLKSCLDNVN
Mcl-1 177 QSLEIISRYLREQATGAKDTKPMGRSGATSRKALETLRRVGDGVQRNHETAFQGMLRKLD
Bcl-2 121 -LTPFTARGRFATVVEELFRDGV-NWGRIVAFFEFGGVMCVESVNREMSPLVD---NIAL
Bcl-W 70 -VTPGSAQQRFTQVSDELFQGGP-NWGRLVAFFVFGAALCAESVNKEMEPLVG---QVQE
Bcl-X 114 -ITPGTAYQSFEQVVNELFRDGV-NWGRIVAFFSFGGALCVESVDKEMQVLVS---RIAA
BFL-1 61 VVSVDTARTLFNQVMEKEFEDGIINWGRIVTIFAFEGILIKKLLRQQIAPDVDTYKEISY
Mcl-1 237 -IKNEDDVKSLSRVMIHVFSDGVTNWGRIVTLISFGAFVAKHLKTINQESCIE---PLAE
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Bcl-W 184 VTVGAFFASK-
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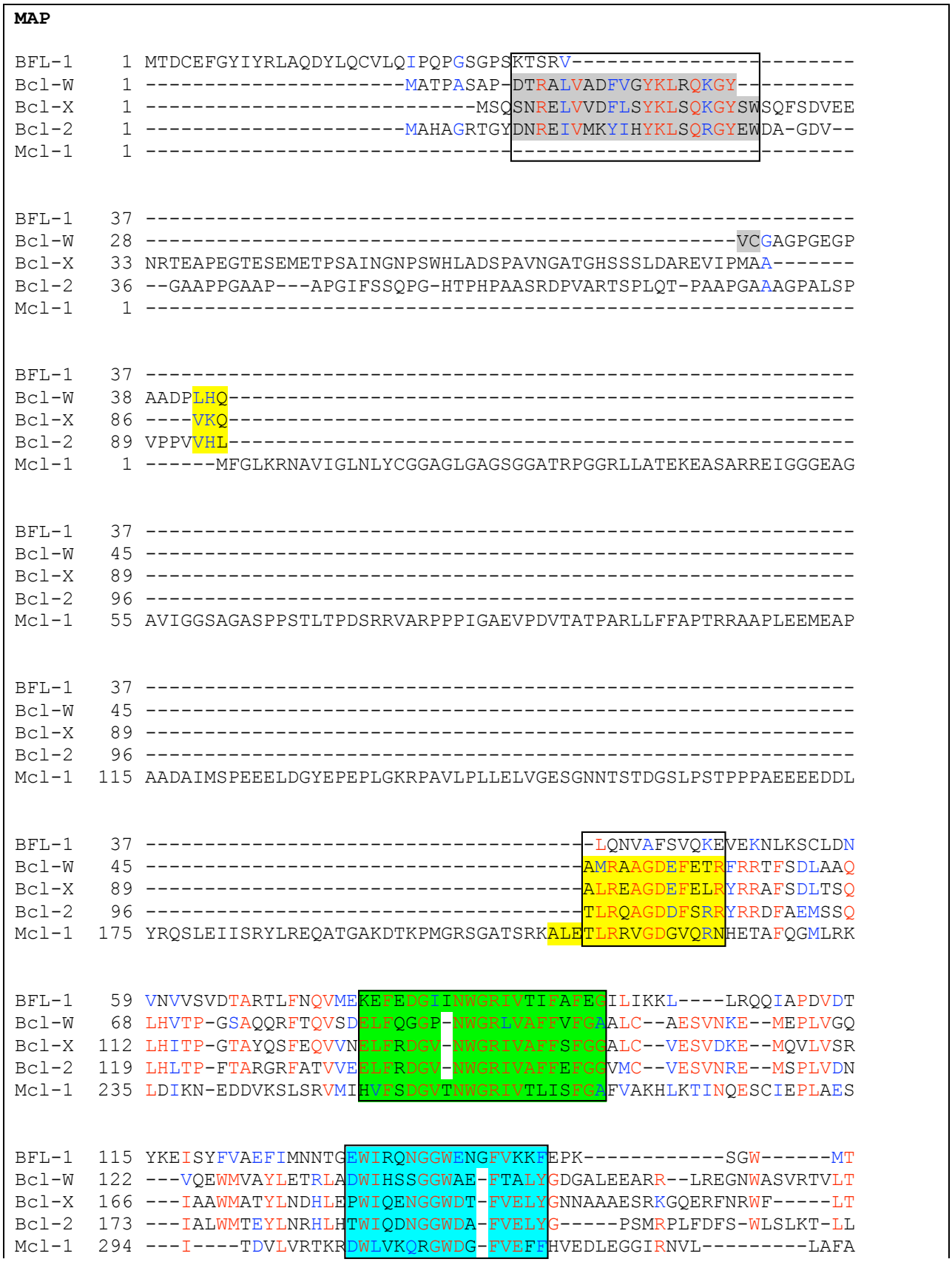
Figure 5C.

MAP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Bcl-W 1 MATPASAP-DTRALVADFVGYKLRQKGY
Bcl-X 1 MSQSNRELVVDFLSYKLSQKGYSWSQFSDVEE
Bcl-2 1 MAHAGRTGYDNREIVMKYIHYKLSQRGYEWDA-GDV
Mcl-1 1
BFL-1 37
Bcl-W 28 VCGAGPGEGP
Bcl-X 33 NRTEAPEGTESEMETPSAINGNPSWHLADSPAVNGATGHSSSLDAREVIPMAA
Bcl-2 36 --GAAPPGAAP---APGIFSSQPG-HTPHPAASRDPVARTSPLQT-PAAPGAAAGPALSP
Mcl-1 1
BFL-1 37
Bcl-W 38 AADPLHQ
Bcl-X 86 VKQ
Bcl-2 89 VPPVVHL
Mcl-1 1 MFGLKRNAVIGLNLYCGGAGLGAGSGGATRPGGRLLATEKEASARREIGGGEAG
BFL-1 37
Bcl-W 45
Bcl-X 89
Bcl-2 96
Mcl-1 55 AVIGGSAGASPPSTLTPDSRRVARPPPIGAEVPDVTATPARLLFFAPTRRAAPLEEMEAP
BFL-1 37
Bcl-W 45
Bcl-X 89
Bcl-2 96
Mcl-1 115 AADAIMSPEEELDGYEPEPLGKRPAVLPLLELVGESGNNTSTDGSLPSTPPPAEEEEDDL
BFL-1 37 LQNVAFSVQKEVEKNLKSCLDN
Bcl-W 45 AMRAAGDEFETRFRRTFSDLAAQ
Bcl-X 89 ALREAGDEFELRYRRAFSDLTSQ
Bcl-2 96 TLRQAGDDFSRRYRRDFAEMSSQ
Mcl-1 175 YRQSLEIISRYLREQATGAKDTKPMGRSGATSRKALETLRRVGDGVQRNHETAFQGMLRK
BFL-1 59 VNVVSVDTARTLFNQVMEKEFEDGIINWGRIVTIFAFGGILIKKL----LRQQIAPDVDT
Bcl-W 68 LHVTP-GSAQQRFTQVSDELFQGGP-NWGRLVAFFVFGAALC--AESVNKE--MEPLVGQ
Bcl-X 112 LHITP-GTAYQSFEQVVNELFRDGV-NWGRIVAFFSFGGALC--VESVDKE--MQVLVSR
Bcl-2 119 LHLTP-FTARGRFATVVEELFRDGV-NWGRIVAFFEFGGVMC--VESVNRE--MSPLVDN
Mcl-1 235 LDIKN-EDDVKSLSRVMIHVFSDGVTNWGRIVTLISFGAFVAKHLKTINQESCIEPLAES
BFL-1 115 YKEISYFVAEFIMNNTGEWIRQNGGWENGFVKKFEPK----------SGW------MT
Bcl-W 122 ---VQEWMVAYLETRLADWIHSSGGWAE-FTALYGDGALEEARR--LREGNWASVRTVLT
Bcl-X 166 ---IAAWMATYLNDHLEPWIQENGGWDT-FVELYGNNAAAESRKGQERFNRWF-----LT
Bcl-2 173 ---IALWMTEYLNRHLHTWIQDNGGWDA-FVELYG-----PSMRPLFDFS-WLSLKT-LL
Mcl-1 294 ---I----TDVLVRTKRDWLVKQRGWDG-FVEFFHVEDLEGGIRNVL---------LAFA

```
BFL-1   157 FLEVTGKICEMLSLLKQYC
Bcl-W   176 GAVALGALVTVGAFFASK-
Bcl-X   217 GMTVAGV-VLLGSLFSRK-
Bcl-2   222 SLALVGACITLGAYLGHK-
Mcl-1   337 GVAGVGAGL---AYLIR--
```

Figure 5D.

ProAlign

```
Mcl1      1 MFGLKRNAVIGLNLYCGGAGLGAGSGGATRPGGRLLATEKEASARREIGGGEAGAVIGGS
BFL1      1 ----------------------M-------------------------------------
BclW      1 ----------------------MA------------------------------------
BclX      1 ----------------------MSQS------NRELVVDFLSYKLSQKGYSWSQ----FS
Bcl-2     1 ----------------------MAHAGRTGYDNREIVMKYIHYKLSQRGYEWDA----GD

Mcl1     61 AGASPPSTLTPDSRRVARPPPIGAEVPDVTATPARLLFFAPTRRAAPLEEMEAPAADAIM
BFL1      2 ------TD--------CEF-G-----YIYRLAQDYLQ--CVLQ----IPQP-GSGPSK--
BclW      3 ------TP--------ASA-P-----DTRALVADFVG--YKLRQKGYVCGA-GPGEGP--
BclX     29 DVEENRTE--------APE-GTESEMETPSAINGNPS--WHLADSPAVNGATGHSSSL--
Bcl-2    35 VGAAPPGA--------APAPGIFSSQPGHTPHPAASR--DPVARTSPLQTPAAPGAAA--

Mcl1    121 SPEEELDGYEPEPLGKRPAVLPLLELVGESGNNTSTDGSLPSTPPPAEEEEDDLYRQSLE
BFL1     33 ----------TSR------VLQN--------------------VAFSVQKEVEKNLKSCLD
BclW     38 ----------AAD-------PLHQ--------------------AMRAAGDEFETRFRRTFS
BclX     76 ----------DAREVIPMAAVKQ--------------------ALREAGDEFELRYRRAFS
Bcl-2    83 ----------GPALSPVPPVVHL--------------------TLRQAGDDFSRRYRRDFA

Mcl1    181 IISRYLREQATGAKDTKPMGRSGATSRKALETLRRVGDGVQRNHETAFQGMLRKLDIKNE
BFL1     58 NVNVVSV--------DTARTLFNQVME--KEFED---GIINWGRIVTI------FAFEGI
BclW     63 DLAAQLH--------VTPGSAQQRFTQVSDELFQ---GGPNWGRLVAF------FVFGAA
BclX    107 DLTSQLH--------ITPGTAYQSFEQVVNELFR---DGVNWGRIVAF------FSFGGA
Bcl-2   114 EMSSQLH--------LTPFTARGRFATVVEELFR---DGVNWGRIVAF------FEFGGV

Mcl1    241 DDVKSLSRVMIHVFSDGVTNWGRIVTLISFGAFVAKHLKTINQESCIEPLAESITDVLVR
BFL1     99 LIKKLLRQQIAPDVDT---------YKE--ISYF---VAEFIMNNTG----EWIRQNGGW
BclW    106 LCAESVNKEMEPLVGQ---------VQEWMVAYLETRLADWIHSSGGWAEFTALYGDGAL
BclX    150 LCVESVDKEMQVLVSR---------IAAWMATYLNDHLEPWIQENGGWDTFVELYGNNAA
Bcl-2   157 MCVESVNREMSPLVDN---------IALWMTEYLNRHLHTWIQDNGGWDAFVELYGPSMR

Mcl1    301 TKRDWLVKQRGWDGFVEFFHVEDLEGGIRNVLLAFAGVAGVGAGLAYLIR
BFL1    141 ENGFVKKFEPKSG-----------WMTFLEVTGKICEMLSLLKQYC----
BclW    157 EEARRLREGNWAS-----------VRTVLTGAVALGALVTVGAFFASK--
BclX    201 AES---RKGQERF-----------NRWFLTG-MTVAGVVLLGSLFSRK--
Bcl-2   208 P----LFDFSWLS-----------LKTLLSLAL-VGACITLGAYLGHK--
```

Figure 5E.

MultAlin

```
BAK      1 MASGQGPGPPRQECGEPALPSASEEQVAQ.....................DTEEVFRSYVF
Bcl-2    1 MAHAGRTGYDNREIVMKYIHYKLSQRGYEWDAGDVGAAPPGAAPAPGIFSSQPGHTPHPA
Bcl-X    1 ......MSQSNRELVVDFLSYKLSQKGYSWS..QFSDVEENRTEAPEGTESEMETPSAIN
Bcl-W    1 MATPA.SAPDTRALVADFVGYKLRQKGY  ..............................
BAX      1 ..................MDGSGEQPRGGGPT.....................SSEQIMKTGAL
BFL-1    1 ..........................................................
BIK      1 ..........................................................
BAD      1 ..................MFQIPEFEPSEQ.....................EDS..SSAER
BID      1 MDCEVNNGSSLRDECITNLLVFGFLQSCS.....................DNS.FRRELD
BIM      1 MAKQPSDVSSECDREGRQLQPAERPPQLR.....................PGAPTSLQTE
HRK      1 ..........................................................


BAK     41 YRHQQEQEAEGVAAPADPEMVTLPLQPSSTMGQVGRQLAIIGDDINRRYDSEFQTMLQHL
Bcl-2   61 ASRDPVARTSPLQTPAAPGAAAGPALSPVP.PVVHLTLRQAGDDFSRRYRRDFAEMSSQL
Bcl-X   53 GNPSWHLADSPAVNGATGHSSSLDAREVIPMAAVKQALREAGDEFELRYRRAFSDLTSQL
Bcl-W   28 ............VCGAGPGE..GPAADPL.....HQAMRAAGDEFETRFRRTFSDLAAQL
BAX     26 LLQGFIQDRAGRMGGEAPELALDPVPQDASTKKLSECLKRIGDELDS..NMELQRMIAAV
BFL-1    1 .MTDCEFGYIYRLAQDYLQCVLQIPQPGSGPSKTSRVLQNVAFSVQKEVEKNLKSCLDNV
BIK      1 ............MSEVRPLSRDILMETLLYEQLLEPPTMEVLGMTDSEEDLDPMEDFDSL
BAD     21 GLGPSPAGDGPSGSGKHHRQAPGLLWDASHQQEQPTSSSHHGGAGAVEIRSRHSSYPAGT
BID     39 ALGHELPVLAPQWEGYDELQTDGNR..SSHSRLGRIEADSESQEDIIRNIARHLAQVGD.
BIM     40 PQGNPEGNHGGEGDSCPHGSPQGPL..APPASPGPFATRSPLFIFMRRSSLLSRSSSGYF
HRK      1 .MCPCPLHRGRGPPAVCACSAGRLGLRSSAAQLTAARLKALGDELHQRTMWRRRARSRRA


BAK    101 QPTA.ENAYEYFTKIATSLFESG.INWGRVVALLGFGYRLALHVYQHGLTGFLGQ...VT
Bcl-2  120 HLTP.FTARGRFATVVEELFRDG.VNWGRIVAFFEFGGVMCVESVNREMSPLVDN...IA
Bcl-X  113 HITP.GTAYQSFEQVVNELFRDG.VNWGRIVAFFSFGGALCVESVDKEMQVLVSR...IA
Bcl-W   69 HVTP.GSAQQRFTQVSDELFQGG.PNWGRLVAFFVFGAALCAESVNKEMEPLVGQ...VQ
BAX     84 D..T.DSPREVFFRVAADMFSDGNFNWGRVVALFYFASKLVLKALCTKVPELIRT...IM
BFL-1   60 NVVSVDTARTLFNQVMEKEFEDGIINWGRIVTIFAFEGILIKKLLRQQIAPDVDTYKEIS
BIK     49 ECMEGSDALALRLACIGDEM.DVSLRAPRLAQLSEVAMHSLGLAFIYDQTEDIRDVLRSF
BAD     81 EDDEGMGEEPSPFRGRSRSAPPNLWAAQRYGRELRRMSDEFVDSFKKGLPRPKS......
BID     96 SMDRSI..PPGLVNGLALQLRNTSRSEEDRNRDLATALEQLLQAYPRDMEKEKT.....M
BIM     98 SFDTDR..SPAPMS.CDKSTQTPSPPCQAFNHYLS.AMASMRQAEPADMRPE........
HRK     60 PAPGALPTYWPWLCAAAQVAALAAWLLGRRNL.............................


BAK    156 RFVVDFMLHHCIARWIAQR.GGWV.AALNL.GNGPILNV.............LVVLGVVLL
Bcl-2  175 LWMTEYLNRH.LHTWIQDN.GGWD.AFVELYGPSMRPLFDF....SWLSL.KTLLSLALV
Bcl-X  168 AWMATYLNDH.LEPWIQEN.GGWD.TFVELYGNNAAAESRK....GQERFNRWFLTGMTV
Bcl-W  124 EWMVAYLETR.LADWIHSS.GGWA.EFTALYGDGALEEARRLREGNWASVRTVLTGAVAL
BAX    138 GWTLDFLRER.LLGWIQDQ.GGWD.GLLSYFGTPTWQTV.............TIFVAGVLT
BFL-1  120 YFVAEFIMNN.TGEWIRQN.GGWENGFVKKFEPKSGWMT.............FLEVTGKIC
BIK    108 MDGFTTLKENIMRFWRSPNPGSWVSCEQVLLALLLL................LALLLPLLS
BAD    135 AGTATQMRQ..SSSWTRVFQSWWDRNLGRGSSAP..................SQ.......
BID    149 LVLALLLAKKVASHTPSLLRDVFHTTVNFINQNL..................RTYVRSLAR
BIM    146 IWIAQELRRIGDEFNAYYARRVFLNNYQAAEDHP..................RMVILRLLR
HRK        ............................................................


BAK    201 GQFV.VRRFFKS.
Bcl-2  227 GACITLGAYLGHK
Bcl-X  221 AGVVLLGSLFSRK
```

```
Bcl-W  181 GALVTVGAFFASK
BAX    183 ASLTIWKKMG...
BFL-1  166 EMLSLLKQYC...
BIK    153 GGLHLLLK.....
BAD        ............
BID    192 NGMD.........
BIM    189 YIVRLVWRMH...
HRK        ............
```

Figure 6A.

ClustalW

```
Bcl-W     1 ---------------------------------------------------MATPASAPD
Bcl-X     1 ------MSQSNRELVVDFLSYKLSQKGYSWSQFSDVEENRTEAPEG-TESEMETPSAING
Bcl-2     1 MAHAGRTGYDNREIVMKYIHYKLSQRGYEWDAGDVGAAPPGAAPAPGIFSSQPGHTPHPA
BAK       1 ---------MASGQGPGPPRQECGEPALPSASEEQVAQDTEEVFRSYVFY
BAX       1 ---------------------------MDGSGEQPRGGGPTS---SEQIMKTGALL
BFL-1     1 ------------------------------------------------------------
HRK       1 ------------------------------------------------------------
BIK       1 ------------------------------------------------------------M
BID       1 ---------------------------------------------MDCEVNNGSSLRD
BAD       1 ------------------------------------------------------------M
BIM       1 ---------------------------------------------------MAKQPSDV

Bcl-W    10 TRALVADFVGYKLRQKGYVCGAGPGEGPAADPLHQAMRAAGDEFETRFRRTFSDLAAQLH
Bcl-X    54 NPSWHLADSPAVNGATGHSSSLDAREVIPMAAVKQALREAGDEFELRYRRAFSDLTSQLH
Bcl-2    61 ASRDPVARTSPLQTPAAPGAAAGPALSPVPPVVHLTLRQAGDDFSRRYRRDFAEMSSQLH
BAK      42 RHQQEQEAEGVAAPADPEMVTLPLQPSSTMGQVGRQLAIIGDDINRRYDSEFQTMLQHLQ
BAX      27 LQGFIQDRAGRMGGEAPELALDPVPQDASTKKLSECLKRIGDELDS--NMELQRMIAAVD
BFL-1     1 MTDCEFGYIYRLAQDYLQCVLQIPQPGSGPSKTSRVLQNVAFSVQKEVEKNLKSCLDNVN
HRK       1 MCPCPLHRGRGPPAVCACSAGRLGLRSSAAQLTAARLKALGDELHQRTMWRRRARSRRAP
BIK       2 SEVRPLSRDILMETLLYEQLLEPPTMEVLGMTDSEEDLDPMEDFDSLECMEGSDALALRL
BID      14 ECITNLLVFGFLQSCSDNSFRRELDALGHELPVLAPQWEGYDELQTDGNRSSHSRLGRIE
BAD       2 FQIPEFEPSEQEDSSSAERGLGPSPAGDGPSGSGKHHRQAPGLLWDASHQQEQPTSSSHH
BIM       9 SSECDREGRQLQPAERPPQLRPGAPTSLQTEPQGNPEGNHGGEGDSCPHGSPQGPLAPPA

Bcl-W    70 VTPG-SAQQRFTQVSDELFQGC-PNWGRLVAFFVFGAALCAESVNKEMEPLVGQVQEWMV
Bcl-X   114 ITPG-TAYQSFEQVVNELFRDG-VNWGRIVAFFSFGGALCVESVDKEMQVLVSRIAAWMA
Bcl-2   121 LTPF-TARGRFATVVEELFRDG-VNWGRIVAFFEFGGVMCVESVNREMSPLVDNIALWMT
BAK     102 PTAE-NAYEYFTKIATSLFESG-INWGRVVALLGFGYRLALHVYQHGLTGFLGQVTRFVV
BAX      85 TD---SPREVFFRVAADMFSDGNFNWGRVVALFYFASKLVLKALCTKVPELIRTIMGWTL
BFL-1    61 VVSVDTARTLFNQVMEKEFEDGIINWGRIVTIFAFEGILIKKLLRQQIAPDVDTYKEISY
HRK      61 APG--ALPTYWPWLCAAAQVAALAAWLLGRRNL----------------------------
BIK      62 ACIG--DEMDVSLRAPRLAQLSEVAMHSLGLAFIYDQTEDIRDVLRSFMDGFTTLKENIM
BID      74 ADSE-SQEDIIRNIARHLAQVGDSMDRSIPPGLVNGLALQLRNTSRSEEDRNRDLATALE
BAD      62 GGAG--AVEIRSRHSSYPAGTEDDEGMGEEPSPFRGRSRSAPPNLWAAQRYGRELRRMSD
BIM      69 SPGPFATRSPLFIFMRRSSLLSRSSSGYFSFDTDRSPAPMSCDKSTQTPSPPCQAFNHYL

Bcl-W   128 AYLETRLAD---WIHSSGGWAEFTALYGDGALEEARRLRE--GNWASVRTVLTGAVALGA
Bcl-X   172 TYLNDHLEP---WIQENGGWDTFVELYGNNAAAESRKGQERFNRWFLTGMTVAGVVLLGS
Bcl-2   179 EYLNRHLHT---WIQDNGGWDAFVELYG----PSMRPLFD--FSWLSLKTLLS-LALVGA
BAK     160 DFMLHHCIAR--WIAQRGGWVAALNLGN---------------GPILNVLVVLGVVLLGQ
BAX     142 DFLRERLLG---WIQDQGGWDGLLSYFG-------------TPTWQTVTIFVAGVLTASL
BFL-1   121 FVAEFIMNNTGEWIRQNGGWENGFVKKFE---------P--KSGWMTFLEVTGKICEMLS
HRK         ----------------------------------------------------------
BIK     120 RFWRSPNPGS--WVSCEQVLLALLLLLA-----------------LLLPLLSGGLHLLLK
BID     133 QLLQAYPRD----MEKEKTMLVLALLLAKKVASHTPSLLRDVFHTTVNFINQNLRTYVRS
BAD     120 EFVDSFKKG---LPRPKSAGTATQMRQS--------------SSWTRVFQSWWDRNLGRG
BIM     129 SAMASMRQAEPADMRPEIWIAQELRRIGDEFNAYYARRVFLNNYQAAEDHPRMVILRLLR
```

```
Bcl-W  183 LVTVGAFFASK
Bcl-X  229 LFSRK------
Bcl-2  229 CITLGAYLGHK
BAK    203 FVVRRFFKS--
BAX    186 TIWKKMG----
BFL-1  170 LLKQYC-----
HRK        ----------
BIK    161 ----------
BID    189 LARNGMD----
BAD    163 SSAPSQ-----
BIM    189 YIVRLVWRMH-
```

Figure 6B.

T-Coffee

```
BAK      1 ------------------------------------------------------MASGQG
BAX      1 -------------------M------------------------------DGSGEQPRG
Bcl-2    1 ------------------------------------------------------------
Bcl-W    1 ------------------------------------------------------------
Bcl-X    1 ------------------------------------------------------------
BFL-1    1 --MTDCEFGYIYRLAQDYLQ-----------------------------CVLQIPQPG
BAD      1 MFQI-----------------------------------------PEFEPSEQEDSSSAERG
BID      1 ---MDCEVNNGSSL-------------------------------------------------
BIK      1 -------MSEVRPLSRDIL-------------------------------METLLYEQL
BIM      1 MAKQPSDVSSECDREGRQLQPAERPPQLRPGAPTSLQTEPQGNPEGNHGGEGDSCPHGSP
HRK      1 ---------------------------------------------------MCPCPLHRG


BAK      7 PGPPRQECGEPALPSASEEQVAQDTEEVFRSYVFYR-----HQQEQE-------AEGVAAP
BAX     11 GGPTS-------------------SEQIMKTGALL-----L--QGFIQD---RAGRMGG
Bcl-2    1 --------------MAHAGRTGYDNREIVMKYIHYK-----LSQRGYEWDAGDVGAAPPG
Bcl-W    1 ---------------MATPASAPDTRALVADFVGYK-----LRQKGYV------CGAGPG
Bcl-X    1 -------------------MSQSNRELVVDFLSYK-----LSQKGYSWSQ---------
BFL-1   28 SGPSK-------------------TSRVLQNVAFS-----V-------------------
BAD     22 LGP------SPAGD-------GPSGSGKHHRQAPGLLWDASHQQEQ---------PTSSSH
BID     12 ----RDECITNLLVFGFLQSCSDNSFRRELDALGHE-----LPVLAPQWEG---------
BIK     22 LEPPTME-----------------------------------------------VLGM
BIM     61 QGPL----APPASP-------GPFAT-----------------------------RSPL
HRK     10 RGPP-------------------------------------------------------


BAK     56 ADP--------------------------------EMVTLPLQ---------PSSTMG---
BAX     41 EAP------------------------------------------------------------
Bcl-2   42 AAPAP----------GIFSSQPGHTPHPAASRDPVARTSPLQ---------TPAAPGAAA
Bcl-W   35 EGP------------------------------------------------------------
Bcl-X   27 ---------------FSDVEENRTEAPEGTESEMETPSAINGNPSWHLADSPAVNGATG
BFL-1   45 ------------------------------------------------------------
BAD     61 HGGAGAVEIRSRHSSYPAGTEDDEGMGEEPSPFRGRSRSAP------------------
BID     54 ----------------YDELQTDGNRSSHSRLGRIEA----------------------
BIK     33 TDS-------------------------EEDLDPMEDFDS-------------LECMEGS
BIM     80 FIFMRRSSLLSRSSSGYFSFDTDRSPAPMSCDKSTQTPSPPCQAFNHYLS----AMASMR
HRK     14 ---------------------------------------------------AVCACSA
```

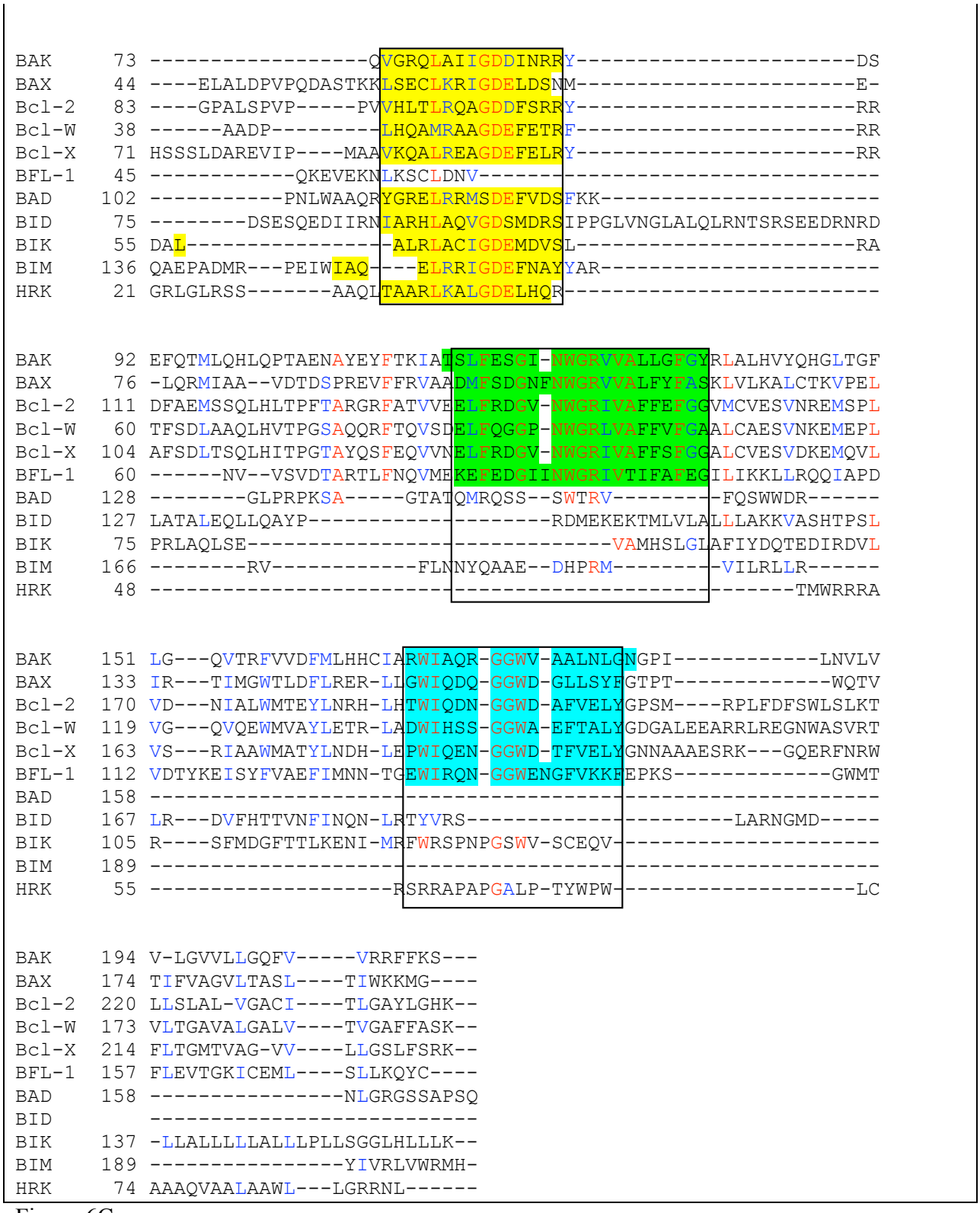

BAK 73 ----------QVGRQLAIIGDDINRRY----------DS
BAX 44 ----ELALDPVPQDASTKKLSECLKRIGDELDSNM----------E-
Bcl-2 83 ----GPALSPVP-----PVVHLTLRQAGDDFSRRY----------RR
Bcl-W 38 ------AADP---------LHQAMRAAGDEFETRF----------RR
Bcl-X 71 HSSSLDAREVIP----MAAVKQALREAGDEFELRY----------RR
BFL-1 45 ------------QKEVEKNLKSCLDNV-------------------
BAD 102 -----------PNLWAAQRYGRELRRMSDEFVDSFKK----------
BID 75 --------DSESQEDIIRNIARHLAQVGDSMDRSIPPGLVNGLALQLRNTSRSEEDRNRD
BIK 55 DAL----------ALRLACIGDEMDVSL----------RA
BIM 136 QAEPADMR---PEIWIAQ----ELRRIGDEFNAYYAR----------
HRK 21 GRLGLRSS-------AAQLTAARLKALGDELHQR----------
BAK 92 EFQTMLQHLQPTAENAYEYFTKIATSLFESGI-NWGRVVALLGFGYRLALHVYQHGLTGF
BAX 76 -LQRMIAA--VDTDSPREVFFRVAADMFSDGNFNWGRVVALFYFASKLVLKALCTKVPEL
Bcl-2 111 DFAEMSSQLHLTPFTARGRFATVVEELFRDGV-NWGRIVAFFEFGGVMCVESVNREMSPL
Bcl-W 60 TFSDLAAQLHVTPGSAQQRFTQVSDELFQGGP-NWGRLVAFFVFGAALCAESVNKEMEPL
Bcl-X 104 AFSDLTSQLHITPGTAYQSFEQVVNELFRDGV-NWGRIVAFFSFGGALCVESVDKEMQVL
BFL-1 60 ------NV--VSVDTARTLFNQVMEKEFEDGII-NWGRIVTIFAFEGILIKKLLRQQIAPD
BAD 128 --------GLPRPKSA-----GTATQMRQSS--SWTRV---------FQSWWDR------
BID 127 LATALEQLLQAYP-----------------RDMEKEKTMLVLALLLAKKVASHTPSL
BIK 75 PRLAQLSE-----------------------------VAMHSLGLAFIYDQTEDIRDVL
BIM 166 --------RV-----------FLNNYQAAE--DHPRM---------VILRLLR------
HRK 48 ---TMWRRRA
BAK 151 LG---QVTRFVVDFMLHHCIARWIAQR-GGWV-AALNLGNGPI----------LNVLV
BAX 133 IR---TIMGWTLDFLRER-LLGWIQDQ-GGWD-GLLSYFGTPT----------WQTV
Bcl-2 170 VD---NIALWMTEYLNRH-LHTWIQDN-GGWD-AFVELYGPSM----RPLFDFSWLSLKT
Bcl-W 119 VG---QVQEWMVAYLETR-LADWIHSS-GGWA-EFTALYGDGALEEARRLREGNWASVRT
Bcl-X 163 VS---RIAAWMATYLNDH-LEPWIQEN-GGWD-TFVELYGNNAAAESRK---GQERFNRW
BFL-1 112 VDTYKEISYFVAEFIMNN-TGEWIRQN-GGWENGFVKKFEPKS----------GWMT
BAD 158 ----------
BID 167 LR---DVFHTTVNFINQN-LRTYVRS----------LARNGMD-----
BIK 105 R----SFMDGFTTLKENI-MRFWRSPNPGSWV-SCEQV----------
BIM 189 ----------
HRK 55 ----------RSRRAPAPGALP-TYWPW----------LC
BAK 194 V-LGVVLLGQFV-----VRRFFKS---
BAX 174 TIFVAGVLTASL----TIWKKMG----
Bcl-2 220 LLSLAL-VGACI----TLGAYLGHK--
Bcl-W 173 VLTGAVALGALV----TVGAFFASK--
Bcl-X 214 FLTGMTVAG-VV----LLGSLFSRK--
BFL-1 157 FLEVTGKICEML----SLLKQYC----
BAD 158 ----------NLGRGSSAPSQ
BID ----------
BIK 137 -LLALLLLLALLLPLLSGGLHLLLK--
BIM 189 ----------YIVRLVWRMH-
HRK 74 AAAQVAALAAWL---LGRRNL------

Figure 6C.

MAP

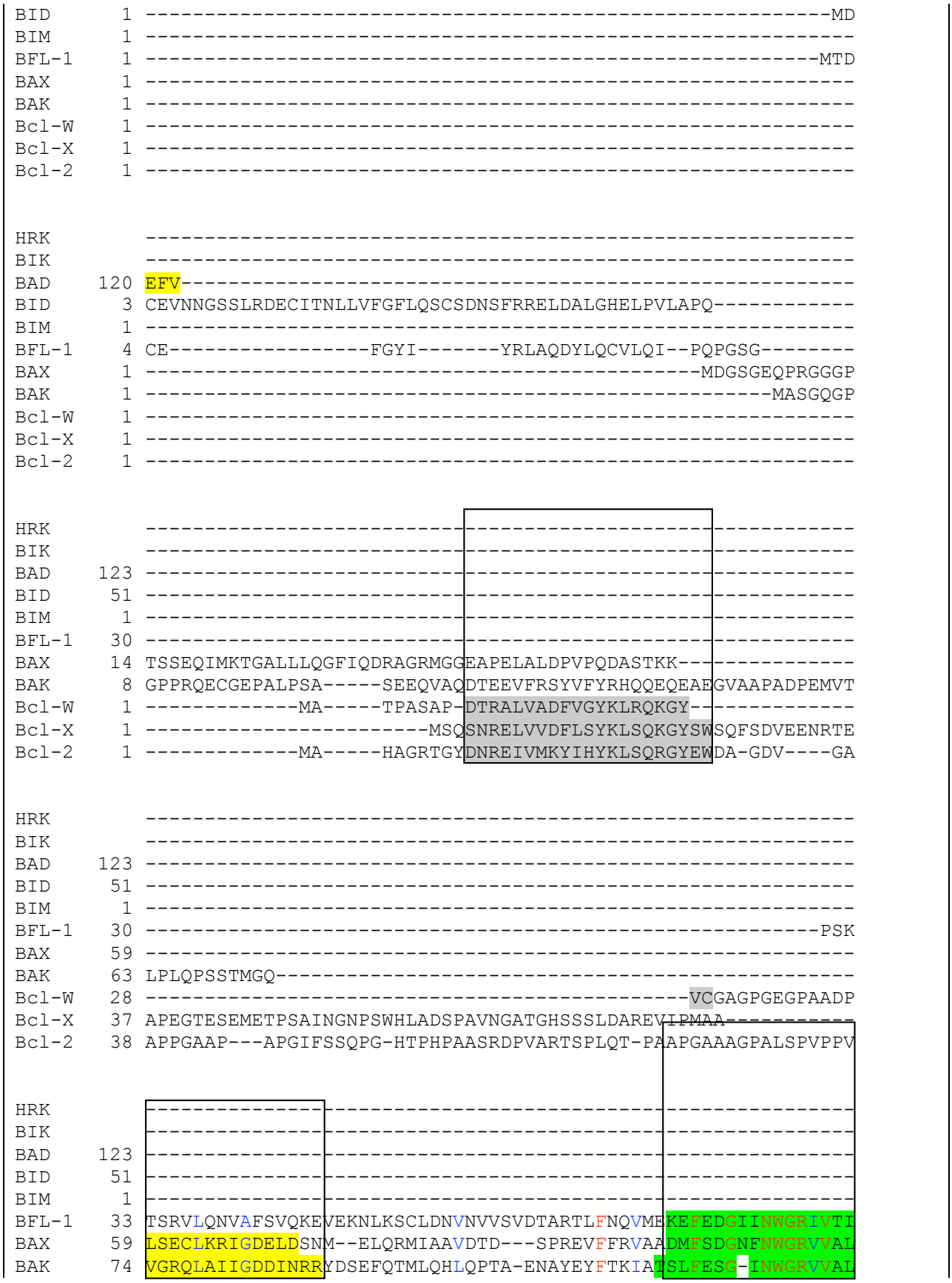
```
HRK      1 ------------------------------------------------------------
BIK      1 MSEVRPLSRDILMETLLYEQLLEPPTMEVLGMTDSEEDLDPMEDFDSLECMEGSDALALR
BAD      1 ------------------------------------------------------------
BID      1 ------------------------------------------------------------
BIM      1 ------------------------------------------------------------
BFL-1    1 ------------------------------------------------------------
BAX      1 ------------------------------------------------------------
BAK      1 ------------------------------------------------------------
Bcl-W    1 ------------------------------------------------------------
Bcl-X    1 ------------------------------------------------------------
Bcl-2    1 ------------------------------------------------------------


HRK      1 ------------------------------------------------------------
BIK     61 LACIGDEMDVSLRAPRLAQLSEVAMHSLGLAFIYDQTEDIRDVLRSFMDGFTTLKENIMR
BAD      1 ------------------------------------------------------------
BID      1 ------------------------------------------------------------
BIM      1 ------------------------------------------------------------
BFL-1    1 ------------------------------------------------------------
BAX      1 ------------------------------------------------------------
BAK      1 ------------------------------------------------------------
Bcl-W    1 ------------------------------------------------------------
Bcl-X    1 ------------------------------------------------------------
Bcl-2    1 ------------------------------------------------------------


HRK      1 ------------------------------------------------------------MC
BIK    121 FWRSPNPGSWVSCEQVLLALLLLLALLLPLLSGGLHLLLK--------------------
BAD      1 ------------------------------------MFQIPEFEPSEQEDSSSAERGLG
BID      1 ------------------------------------------------------------
BIM      1 ------------------------------------------------------------
BFL-1    1 ------------------------------------------------------------
BAX      1 ------------------------------------------------------------
BAK      1 ------------------------------------------------------------
Bcl-W    1 ------------------------------------------------------------
Bcl-X    1 ------------------------------------------------------------
Bcl-2    1 ------------------------------------------------------------


HRK      3 PCPLHRGRGP----------------------PAVCAC--SAGRLGLRSSAAQLTAARL
BIK        ------------------------------------------------------------
BAD     24 PSPA--GDGPSGSGKHHRQAPGLLWDASHQQEQPTSSSHHGGAGAVEIRSRHSSYPAGTE
BID      1 ------------------------------------------------------------
BIM      1 ------------------------------------------------------------
BFL-1    1 ------------------------------------------------------------
BAX      1 ------------------------------------------------------------
BAK      1 ------------------------------------------------------------
Bcl-W    1 ------------------------------------------------------------
Bcl-X    1 ------------------------------------------------------------
Bcl-2    1 ------------------------------------------------------------


HRK     38 --KALGDELHQRTMWRRRARSRRAPAPGALPTYWPWLCAAAQVAALAAWLLGR--RNL--
BIK        ------------------------------------------------------------
BAD     82 DDEGMGEEPSPF-----RGRSRSAP-----PNLW-----------AAQRYGRELRRMSD
```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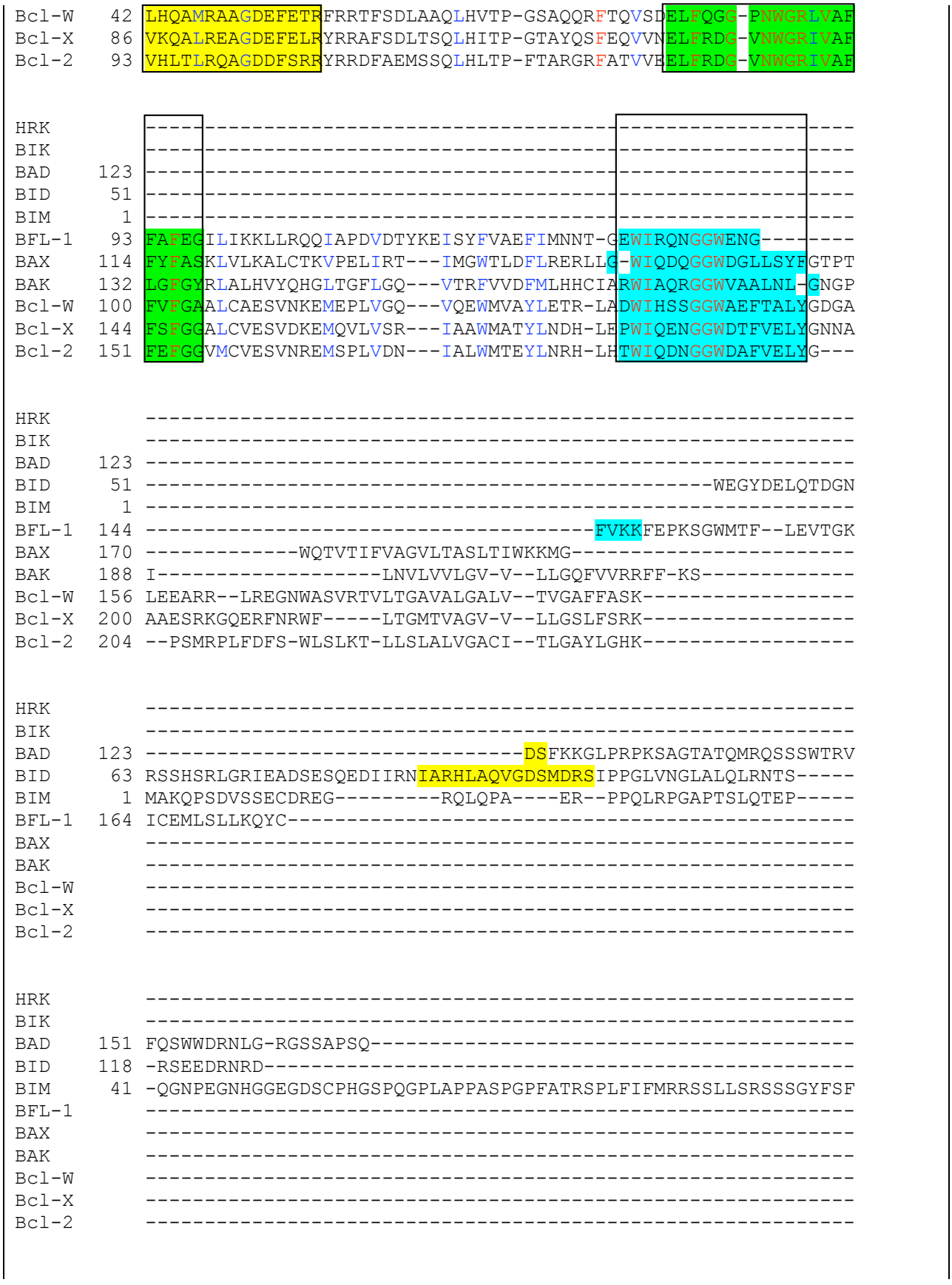
BIM 1 ----------
BFL-1 1 ----------MTD
BAX 1 ----------
BAK 1 ----------
Bcl-W 1 ----------
Bcl-X 1 ----------
Bcl-2 1 ----------
HRK ----------
BIK ----------
BAD 120 EFV----------
BID 3 CEVNNGSSLRDECITNLLVFGFLQSCSDNSFRRELDALGHELPVLAPQ----------
BIM 1 ----------
BFL-1 4 CE----------FGYI-------YRLAQDYLQCVLQI--PQPGSG--------
BAX 1 ----------MDGSGEQPRGGGP
BAK 1 ----------MASGQGP
Bcl-W 1 ----------
Bcl-X 1 ----------
Bcl-2 1 ----------
HRK ----------
BIK ----------
BAD 123 ----------
BID 51 ----------
BIM 1 ----------
BFL-1 30 ----------
BAX 14 TSSEQIMKTGALLLQGFIQDRAGRMGGEAPELALDPVPQDASTKK----------
BAK 8 GPPRQECGEPALPSA-----SEEQVAQDTEEVFRSYVFYRHQQEQEAEGVAAPADPEMVT
Bcl-W 1 ----------MA-----TPASAP-DTRALVADFVGYKLRQKGY----------
Bcl-X 1 ----------MSQSNRELVVDFLSYKLSQKGYSWSQFSDVEENRTE
Bcl-2 1 ----------MA-----HAGRTGYDNREIVMKYIHYKLSQRGYEWDA-GDV----GA
HRK ----------
BIK ----------
BAD 123 ----------
BID 51 ----------
BIM 1 ----------
BFL-1 30 ----------PSK
BAX 59 ----------
BAK 63 LPLQPSSTMGQ----------
Bcl-W 28 ----------VCGAGPGEGPAADP
Bcl-X 37 APEGTESEMETPSAINGNPSWHLADSPAVNGATGHSSSLDAREVIPMAA----------
Bcl-2 38 APPGAAP---APGIFSSQPG-HTPHPAASRDPVARTSPLQT-PAAPGAAAGPALSPVPPV
HRK ----------
BIK ----------
BAD 123 ----------
BID 51 ----------
BIM 1 ----------
BFL-1 33 TSRVLQNVAFSVQKEVEKNLKSCLDNVNVVSVDTARTLFNQVMELKEFEDGIINWGRIVTI
BAX 59 LSECLKRIGDELDSNM--ELQRMIAAVDTD---SPREVFFRVAADMFSDGNFNWGRVVAL
BAK 74 VGRQLAIIGDDINRRYDSEFQTMLQHLQPTA-ENAYEYFTKIATSLFESG-INWGRVVAL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Bcl-X 86 VKQALREAGDEFELRYRRAFSDLTSQLHITP-GTAYQSFEQVVNELFRDG-VNWGRIVAF
Bcl-2 93 VHLTLRQAGDDFSRRYRRDFAEMSSQLHLTP-FTARGRFATVVEELFRDG-VNWGRIVAF
HRK ----------
BIK ----------
BAD 123 ----------
BID 51 ----------
BIM 1 ----------
BFL-1 93 FAFEGILIKKLLRQQIAPDVDTYKEISYFVAEFIMNNT-GEWIRQNGGWENG---------
BAX 114 FYFASKLVLKALCTKVPELIRT---IMGWTLDFLRERLLG-WIQDQGGWDGLLSYFGTPT
BAK 132 LGFGYRLALHVYQHGLTGFLGQ---VTRFVVDFMLHHCIARWIAQRGGWVAALNL-GNGP
Bcl-W 100 FVFGAALCAESVNKEMEPLVGQ---VQEWMVAYLETR-LADWIHSSGGWAEFTALYGDGA
Bcl-X 144 FSFGGALCVESVDKEMQVLVSR---IAAWMATYLNDH-LEPWIQENGGWDTFVELYGNNA
Bcl-2 151 FEFGGVMCVESVNREMSPLVDN---IALWMTEYLNRH-LHTWIQDNGGWDAFVELYG---
HRK ----------
BIK ----------
BAD 123 ----------
BID 51 ----------WEGYDELQTDGN
BIM 1 ----------
BFL-1 144 ----------FVKKFEPKSGWMTF--LEVTGK
BAX 170 ----------WQTVTIFVAGVLTASLTIWKKMG----------
BAK 188 I----------LNVLVVLGV-V--LLGQFVVRRFF-KS----------
Bcl-W 156 LEEARR--LREGNWASVRTVLTGAVALGALV--TVGAFFASK----------
Bcl-X 200 AAESRKGQERFNRWF-----LTGMTVAGV-V--LLGSLFSRK----------
Bcl-2 204 --PSMRPLFDFS-WLSLKT-LLSLALVGACI--TLGAYLGHK----------
HRK ----------
BIK ----------
BAD 123 ----------DSFKKGLPRPKSAGTATQMRQSSSWTRV
BID 63 RSSHSRLGRIEADSESQEDIIRNIARHLAQVGDSMDRSIPPGLVNGLALQLRNTS-----
BIM 1 MAKQPSDVSSECDREG---------RQLQPA----ER--PPQLRPGAPTSLQTEP-----
BFL-1 164 ICEMLSLLKQYC----------
BAX ----------
BAK ----------
Bcl-W ----------
Bcl-X ----------
Bcl-2 ----------
HRK ----------
BIK ----------
BAD 151 FQSWWDRNLG-RGSSAPSQ----------
BID 118 -RSEEDRNRD----------
BIM 41 -QGNPEGNHGGEGDSCPHGSPQGPLAPPASPGPFATRSPLFIFMRRSSLLSRSSSGYFSF
BFL-1 ----------
BAX ----------
BAK ----------
Bcl-W ----------
Bcl-X ----------
Bcl-2 ----------

```
HRK         ------------------------------------------------------------
BIK         ------------------------------------------------------------
BAD         ------------------------------------------------------------
BID     127 -------------------------LATALEQLLQAYPRDMEKEKTMLVLALLLAKKV
BIM     100 DTDRSPAPMSCDKSTQTPSPPCQAFNHYLSAMASMRQAEPADMRPE---IWIAQELRRIG
BFL-1       ------------------------------------------------------------
BAX         ------------------------------------------------------------
BAK         ------------------------------------------------------------
Bcl-W       ------------------------------------------------------------
Bcl-X       ------------------------------------------------------------
Bcl-2       ------------------------------------------------------------


HRK         ----------------------------------------
BIK         ----------------------------------------
BAD         ----------------------------------------
BID     160 ASHTPSLLRDVFHTTVNFINQNLRTYVRSLARNGMD------
BIM     157 DEFNAYYARRVFLNNYQAAEDHPRMVILRLLRYIVRLVWRMH
BFL-1       ----------------------------------------
BAX         ----------------------------------------
BAK         ----------------------------------------
Bcl-W       ----------------------------------------
Bcl-X       ----------------------------------------
Bcl-2       ----------------------------------------
```

Figure 6D.

ProAlign

```
HRK      1 ----MCPCPLHRGRGPPAV---C------ACSAGRLGLRSSAA-----------------
BIK      1 ---MSEVRPLSRDILMETL---LYEQLLEPPTMEVLGMTDSEE-----------------
BAD      1 ---------|----------------------|---------MFQIPEFEPSEQEDSSSAERG
Bcl2     1 MAHAGRTGYDNREIVMKYIHYKLSQRGYEWDAGDVGAAPPGAAPAPGIFSSQPGHTPHPA
BclX     1 MSQS------NRELVVDFLSYKLSQKGYSWSQFSDVEENRTEAPE-GTESEMETPSAING
BclW     1 MATPA-SAPDTRALVADFVGYKLRQKGYVCGAG------PGEGPA---------------
BAK      1 MASGQGPGPPRQEC---------GEPALPSASEEQVAQDTEEVFRSYVFYRHQQEQEAEG
BFL1     1 -MTDCEFGYIYR-LAQDYLQCVL------|-----------QIPQPG-------------
BAX      1 -MDGSGEQPRGGGPTSSEQIMKTGALLLQGFIQDRAGRMGGEAPE--------------
BIM      1 MAKQPSDVSSECDREGRQLQPAERPPQLRP-----------GAPT-------------S
BID      1 MDCEVNNGSSLRDECITNLL-VFGFLQSCS-----------DNSF-------------R


HRK     31 -QLT----------------------------|-AARLKALGDELHQR-------------
BIK     38 -DLDPMEDFDSLECMEGSDA-----------|LALRLACIGDEMDVSLRAPRLAQLSEVA
BAD     22 LGPSPAGDGPSGSGKHHRQAPGLLWDASHQQEQPTSSSHHGGAGAVEIRSRHSSYPAGTE
Bcl2    61 ASRDPVARTSPLQTPAAPGAAAGPALSPVPPVVHLTLRQAGDDFSRRYRRDFAEMSSQLH
BclX    54 NPSWHLADSPAVNGATGHSSSLDAREVIPMAAVKQALREAGDEFELRYRRAFSDLTSQLH
BclW    39 --ADPL---------------------------HQAMRAAGDEFETRFRRTFSDLAAQLH
BAK     52 VAAP--ADPEMVTLPLQPSSTMGQ--------VGRQLAIIGDDINRRYDSEFQTMLQHLQ
BFL1    28 ------------SGPSKTSRVLQNVAFSVQKEVEKNLKSCLDNVNV-------------
BAX     45 LALDPV--------PQDAST----------KKLSECLKRIGDELDSNM--ELQRMIAA--
BIM     36 LQTEPQGNPEGNHGGEGDSCPHGS------PQGPLAPPASPGPFATRSPLFIFMRRSSLL
BID     35 RELDALGHELPVLAPQWEGYDELQ------TDGNRSSHSRLGRIEADSESQEDIIRNIAR
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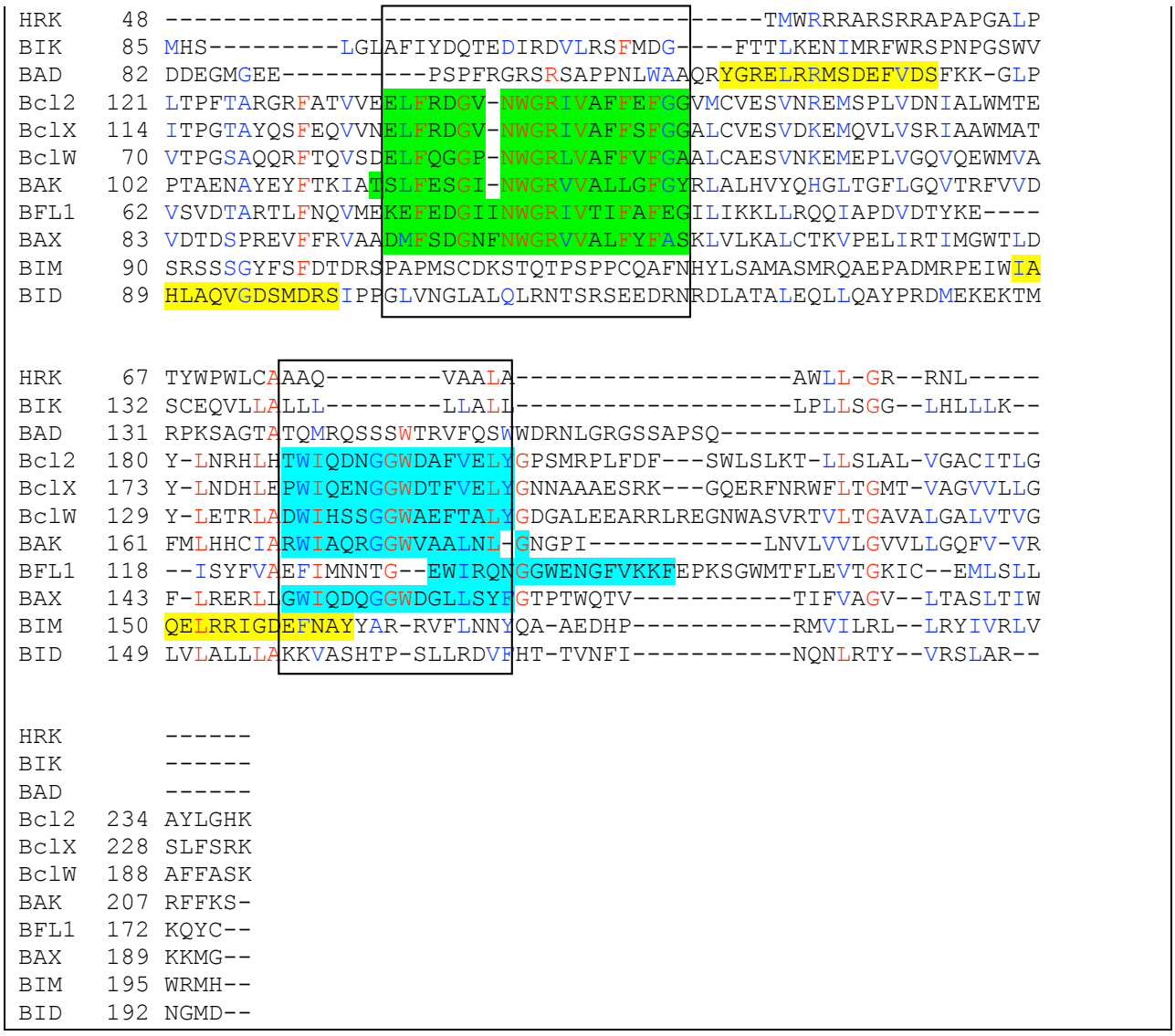
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BAD 82 DDEGMGEE---------PSPFRGRSRSAPPNLWAAQRYGRELRRMSDEFVDSFKK-GLP
Bcl2 121 LTPFTARGRFATVVEELFRDGV-NWGRIVAFFEFGGVMCVESVNREMSPLVDNIALWMTE
BclX 114 ITPGTAYQSFEQVVNELFRDGV-NWGRIVAFFSFGGALCVESVDKEMQVLVSRIAAWMAT
BclW 70 VTPGSAQQRFTQVSDELFQGGP-NWGRLVAFFVFGAALCAESVNKEMEPLVGQVQEWMVA
BAK 102 PTAENAYEYFTKIATSLFESGI-NWGRVVALLGFGYRLALHVYQHGLTGFLGQVTRFVVD
BFL1 62 VSVDTARTLFNQVMEKEFEDGIINWGRIVTIFAFEGILIKKLLRQQIAPDVDTYKE----
BAX 83 VDTDSPREVFFRVAADMFSDGNFNWGRVVALFYFASKLVLKALCTKVPELIRTIMGWTLD
BIM 90 SRSSSGYFSFDTDRSPAPMSCDKSTQTPSPPCQAFNHYLSAMASMRQAEPADMRPEIWIA
BID 89 HLAQVGDSMDRSIPPGLVNGLALQLRNTSRSEEDRNRDLATALEQLLQAYPRDMEKEKTM
HRK 67 TYWPWLCAAAQ--------VAALA--------------------AWLL-GR--RNL-----
BIK 132 SCEQVLLALLL--------LLALL--------------------LPLLSGG--LHLLLK--
BAD 131 RPKSAGTATQMRQSSSWTRVFQSWWDRNLGRGSSAPSQ----------------------
Bcl2 180 Y-LNRHLHTWIQDNGGWDAFVELYGPSMRPLFDF---SWLSLKT-LLSLAL-VGACITLG
BclX 173 Y-LNDHLEPWIQENGGWDTFVELYGNNAAAESRK---GQERFNRWFLTGMT-VAGVVLLG
BclW 129 Y-LETRLADWIHSSGGWAEFTALYGDGALEEARRLREGNWASVRTVLTGAVALGALVTVG
BAK 161 FMLHHCIARWIAQRGGWVAALNL-GNGPI------------LNVLVVLGVVLLGQFV-VR
BFL1 118 --ISYFVAEFIMNNTG--EWIRQNGGWENGFVKKFEPKSGWMTFLEVTGKIC--EMLSLL
BAX 143 F-LRERLLGWIQDQGGWDGLLSYFGTPTWQTV-----------TIFVAGV--LTASLTIW
BIM 150 QELRRIGDEFNAYYAR-RVFLNNYQA-AEDHP-----------RMVILRL--LRYIVRLV
BID 149 LVLALLLAKKVASHTP-SLLRDVFHT-TVNFI-----------NQNLRTY--VRSLAR--
HRK ------
BIK ------
BAD ------
Bcl2 234 AYLGHK
BclX 228 SLFSRK
BclW 188 AFFASK
BAK 207 RFFKS-
BFL1 172 KQYC--
BAX 189 KKMG--
BIM 195 WRMH--
BID 192 NGMD--

Figure 6E.